

# INTERNATIONAL COOPERATION TR

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark  
Office  
(Box PCT)  
Crystal Plaza 2  
Washington, DC 20231  
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)  
15 June 1999 (15.06.99)

International application No.  
PCT/GB98/03483

Applicant's or agent's file reference  
HCM/MJL/GB/C372-02/U

International filing date (day/month/year)  
23 November 1998 (23.11.98)

Priority date (day/month/year)  
21 November 1997 (21.11.97)

Applicant

UNILVER N.V. et al

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
03 May 1999 (03.05.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

PC

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

9/554956

Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum)

## Box No. I TITLE OF INVENTION

Improvements in or Relating to Displacement Assays

## Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

UNILEVER PLC  
Unilever House  
Blackfriars  
London EC4P 4BQ  
United Kingdom

☐ This person is also inventor.Telephone No.  
(01234) 222068Facsimile No.  
(01234) 222633

Teleprinter No.

State (that is, country) of nationality:  
GBState (that is, country) of residence:  
GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☒ the States indicated in the Supplemental Box

## Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

UNILEVER NV  
Weena 455  
3013 AL Rotterdam  
Netherlands

This person is:

☒ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)State (that is, country) of nationality:  
NLState (that is, country) of residence:  
NL

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

## Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒ agent ☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Keith W Nash & Co  
90-92 Regent Street  
Cambridge  
CB2 1DP  
United Kingdom

Telephone No.  
(01223) 355477Facsimile No.  
(01223) 324353

Teleprinter No.

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III **OTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)***If none of the following sub-boxes is used, this sheet should not be included in the request.*

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BADLEY, Robert Andrew  
19 Wellpond Close  
Sharnbrook  
Bedford MK44 1PL  
United Kingdom

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:  
GB

State (that is, country) of residence:  
GB

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

BERRY, Mark John  
2 Fitzwilliam Leys  
Higham Ferrers  
Northamptonshire  
NN10 8LY  
United Kingdom

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:  
GB

State (that is, country) of residence:  
GB

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

HOWELL, Stephen  
10 Heacham Way  
Higham Ferrers  
Northamptonshire  
NN10 8LX

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:  
GB

State (that is, country) of residence:  
GB

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

## Box No.V DESIGNATION OF STATES

The following designations are made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

## Regional Patent

- ☒ **AF** ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

## National Patent (if other kind of protection or treatment desired, specify on dotted line):

- |  |  |
|--|--|
| <input checked="" type="checkbox"/> AL Albania                               | <input checked="" type="checkbox"/> LS Lesotho                                   |
| <input checked="" type="checkbox"/> AM Armenia                               | <input checked="" type="checkbox"/> LT Lithuania                                 |
| <input checked="" type="checkbox"/> AT Austria                               | <input checked="" type="checkbox"/> LU Luxembourg                                |
| <input checked="" type="checkbox"/> AU Australia                             | <input checked="" type="checkbox"/> LV Latvia                                    |
| <input checked="" type="checkbox"/> AZ Azerbaijan                            | <input checked="" type="checkbox"/> MD Republic of Moldova                       |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina                | <input checked="" type="checkbox"/> MG Madagascar                                |
| <input checked="" type="checkbox"/> BB Barbados                              | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria                              |  |
| <input checked="" type="checkbox"/> BR Brazil                                | <input checked="" type="checkbox"/> MN Mongolia                                  |
| <input checked="" type="checkbox"/> BY Belarus                               | <input checked="" type="checkbox"/> MW Malawi                                    |
| <input checked="" type="checkbox"/> CA Canada                                | <input checked="" type="checkbox"/> MX Mexico                                    |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> NO Norway                                    |
| <input checked="" type="checkbox"/> CN China                                 | <input checked="" type="checkbox"/> NZ New Zealand                               |
| <input checked="" type="checkbox"/> CU Cuba                                  | <input checked="" type="checkbox"/> PL Poland                                    |
| <input checked="" type="checkbox"/> CZ Czech Republic                        | <input checked="" type="checkbox"/> PT Portugal                                  |
| <input checked="" type="checkbox"/> DE Germany                               | <input checked="" type="checkbox"/> RO Romania                                   |
| <input checked="" type="checkbox"/> DK Denmark                               | <input checked="" type="checkbox"/> RU Russian Federation                        |
| <input checked="" type="checkbox"/> EE Estonia                               | <input checked="" type="checkbox"/> SD Sudan                                     |
| <input checked="" type="checkbox"/> ES Spain                                 | <input checked="" type="checkbox"/> SE Sweden                                    |
| <input checked="" type="checkbox"/> FI Finland                               | <input checked="" type="checkbox"/> SG Singapore                                 |
| <input checked="" type="checkbox"/> GB United Kingdom                        | <input checked="" type="checkbox"/> SI Slovenia                                  |
| <input checked="" type="checkbox"/> GE Georgia                               | <input checked="" type="checkbox"/> SK Slovakia                                  |
| <input checked="" type="checkbox"/> GH Ghana                                 | <input checked="" type="checkbox"/> SL Sierra Leone                              |
| <input checked="" type="checkbox"/> GM Gambia                                | <input checked="" type="checkbox"/> TJ Tajikistan                                |
| <input checked="" type="checkbox"/> GW Guinea-Bissau                         | <input checked="" type="checkbox"/> TM Turkmenistan                              |
| <input checked="" type="checkbox"/> HR Croatia                               | <input checked="" type="checkbox"/> TR Turkey                                    |
| <input checked="" type="checkbox"/> HU Hungary                               | <input checked="" type="checkbox"/> TT Trinidad and Tobago                       |
| <input checked="" type="checkbox"/> ID Indonesia                             | <input checked="" type="checkbox"/> UA Ukraine                                   |
| <input checked="" type="checkbox"/> IL Israel                                | <input checked="" type="checkbox"/> UG Uganda                                    |
| <input checked="" type="checkbox"/> IS Iceland                               | <input checked="" type="checkbox"/> US United States of America                  |
| <input checked="" type="checkbox"/> JP Japan                                 |  |
| <input checked="" type="checkbox"/> KE Kenya                                 | <input checked="" type="checkbox"/> UZ Uzbekistan                                |
| <input checked="" type="checkbox"/> KG Kyrgyzstan                            | <input checked="" type="checkbox"/> VN Viet Nam                                  |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> YU Yugoslavia                                |
|  | <input checked="" type="checkbox"/> ZW Zimbabwe                                  |
| <input checked="" type="checkbox"/> KR Republic of Korea                     |  |
| <input checked="" type="checkbox"/> KZ Kazakhstan                            |  |
| <input checked="" type="checkbox"/> LC Saint Lucia                           |  |
| <input checked="" type="checkbox"/> LK Sri Lanka                             | <input checked="" type="checkbox"/> GD Grenada                                   |
| <input checked="" type="checkbox"/> LR Liberia                               | <input type="checkbox"/>   |

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

**Precautionary Designation Statement:** In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

**Supplemental Box** If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

#### CONTINUATION OF BOXES NO. II AND NO. III

UNILEVER PLC - Australia, Barbados, Canada, Gambia, Gambia (ARIPO State), Ghana, Ghana (ARIPO State), Ireland (European State), Israel, Kenya, Kenya (ARIPO State), Lesotho, Lesotho (ARIPO State), Malawi, Malawi (ARIPO State), Mongolia, New Zealand, Saint Lucia, Sierra Leone, Singapore, Sri Lanka, Sudan, Sudan (ARIPO State), Swaziland (ARIPO State), Trinidad & Tobago, Uganda, Uganda (ARIPO State), United Kingdom, United Kingdom (European State), Zimbabwe, Zimbabwe (ARIPO State), Cyprus (European State), Grenada

UNILEVER NV - All designated states except those listed for UNILEVER PLC

<b>Box No. VI PRIORITY CLAIM</b>		<input type="checkbox"/> Further priority indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application: regional Office	international application: receiving Office
item (1) 21/11/97	97309409.7		EP	
item (2)				
item (3)				

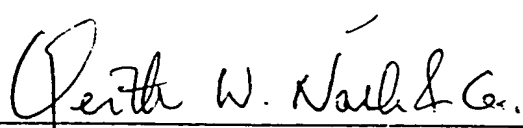
☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

<b>Box No. VII INTERNATIONAL SEARCHING AUTHORITY</b>			
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)	Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority)		
ISA /	Date (day/month/year)	Number	Country (or regional Office)

<b>Box No. VIII CHECK LIST; LANGUAGE OF FILING</b>	
This international application contains the following number of sheets: request : 5 description (excluding sequence listing part) : 27 claims : 4 abstract : 1 drawings : 12 sequence listing part of description : 1 Total number of sheets : 50	This international application is accompanied by the item(s) marked below: 1. <input checked="" type="checkbox"/> fee calculation sheet 2. <input checked="" type="checkbox"/> separate signed power of attorney 3. <input type="checkbox"/> copy of general power of attorney; reference number, if any: 4. <input type="checkbox"/> statement explaining lack of signature 5. <input checked="" type="checkbox"/> priority document(s) identified in Box No. VI as item(s): 6. <input type="checkbox"/> translation of international application into (language): 7. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material 8. <input checked="" type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form 9. <input type="checkbox"/> other (specify):

Figure of the drawings which should accompany the abstract: 1	Language of filing of the international application: English
---	--

<b>Box No. IX SIGNATURE OF APPLICANT OR AGENT</b>	
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).	
 Keith W Nash & Co (Agents)	

For receiving Office use only	
1. Date of actual receipt of the purported international application:	2. Drawings:  <input type="checkbox"/> received:  <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	
6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.	

For International Bureau use only	
Date of receipt of the record copy by the International Bureau:	

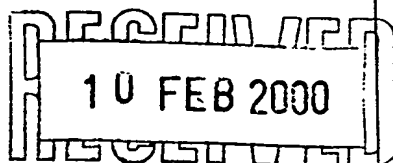
## PATENT COOPERATION TREATY

09 / 554956

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Keith W Nash & Co  
90-92 Regent Street  
Cambridge CB2 1DP  
GRANDE BRETAGNE



PCT

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT

(PCT Rule 71.1)

PTO/PCT Rec'd 22 MAY 2000

Date of mailing  
(day/month/year)

09.02.00

Applicant's or agent's file reference  
MJL/C372-02/U

## IMPORTANT NOTIFICATION

International application No.  
PCT/GB98/03483

International filing date (day/month/year)  
23/11/1998

Priority date (day/month/year)  
21/11/1997

Applicant  
UNILEVER PLC et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

## 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

 European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d  
Fax: +49 89 2399 - 4465

Authorized officer

Digiusto, M


Tel. +49 89 2399-8162



# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>MJL/C372-02/U</b>		<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/GB98/03483</b>	International filing date (day/month/year) <b>23/11/1998</b>	Priority date (day/month/year) <b>21/11/1997</b>	
International Patent Classification (IPC) or national classification and IPC <b>G01N33/543</b>			
Applicant <b>UNILEVER PLC et al.</b>			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 3 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the report</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI <input checked="" type="checkbox"/> Certain documents cited</li> <li>VII <input checked="" type="checkbox"/> Certain defects in the international application</li> <li>VIII <input checked="" type="checkbox"/> Certain observations on the international application</li> </ul>			
Date of submission of the demand  <b>03/05/1999</b>		Date of completion of this report  <b>09. 02. 00</b>	
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465		Authorized officer  <b>Luis Alves, D</b>  Telephone No. +49 89 2399 8695	





**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB98/03483

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-27 as originally filed

**Claims, No.:**

1-21 as received on 24/12/1999 with letter of 20/12/1999

**Drawings, sheets:**

1/12-12/12 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**see separate sheet**

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.  
☒ claims Nos. 20, 21.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB98/03483

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. as above are so unclear that no meaningful opinion could be formed (*specify*):
- see separate sheet**
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-16, 19
	No:	Claims	17, 18
Inventive step (IS)	Yes:	Claims	1-16, 19
	No:	Claims	17, 18
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

**2. Citations and explanations**

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB98/03483

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**VI. Certain documents cited**

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB98/03483

Reference is made to the following documents cited in the International search report:

D1: WO-A-93/21530

D2: WO-A-97/44664

**Section I:**

Additional observations:

The documents forming the basis of this opinion include sequence listing page 1 as originally filed.

**Section III:**

1. The subject-matter of claims 20 and 21 is totally unclear because no features characterising the claimed methods are defined (Article 6 PCT). Moreover, the wording of said claims is conflicting with the requirements of Rule 6.2(a) PCT. Consequently, no opinion will be established with respect to the novelty, inventive step and industrial applicability of claims 20 and 21 (Article 34(4)(a)(ii) PCT).

**Section V:**

1. D1 discloses a method of determining an analyte in a sample using two surfaces: One onto which an analogue of the analyte is immobilised and another onto which a capture molecule for the analyte is immobilised. The analogue of the analyte is an analyte molecule comprising a marker, such that upon release of the analogue from the first surface and capture on the second surface the marker will generate a detectable signal (see example 6). This example shows determination of an analyte using a first surface coated with a displaceable molecule (marked analyte) which upon application of the sample is captured on the second surface (coated with antibody for the analyte). The fluorescence on the second surface is measured. However, D1 neither discloses nor suggests generation of a signal by the

displaceable molecule only upon capture onto the second surface. The subject-matter of claim 1 and dependent claims 2 to 16 complies with the requirements of Article 33(2) and (3) PCT.

2. D1 discloses surfaces for performing the assay, which may have immobilised thereon antibodies, directly or through spacer molecules (see p.16 third and fourth paragraphs). Thus, the features in claims 17 and 18 are disclosed (Article 33(2) PCT).
3. The feature in claim 19 is neither disclosed nor suggested by any of the cited documents and therefore appears to comply with the requirements of Article 33(2) and (3) PCT.

**Section VI:**

1. Document D2, cited in the International search report as an intermediate document, was published on 27 November 1997, filed on 20 May 1997 and claims a priority date of 23 May 1996. This document may become relevant to the subject-matter of the present claims if the priority date of 21 November 1997 is not validly claimed.

**Section VII:**

1. References to patent application numbers, such as on p.8, line 9, and to internal references, such as on p.13, third paragraph, should be replaced by the corresponding publication numbers.
2. There is a clerical error on claim 8, which refers back to claim 8 instead of claim 7.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/GB98/03483

**Section VIII:**

1. Claim 5 lacks clarity (Article 6 PCT) because the expression "a portion which facilitates reversible immobilisation on the first surface" is vague.
2. The expression "eg mimotope" used in claim 9 introduces a lack of clarity (Article 6 PCT) and should be deleted.

## CLAIMS

1. A method of detecting the presence of an analyte of interest in a sample, the method comprising the steps of: providing a first surface having reversibly immobilised thereon a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface in an affinity-related manner; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein said detection is performed by means other than SPR, and wherein the displaceable moiety cannot generate the signal which is detected in the assay unless and until the displaceable moiety is captured on the second surface.
2. A method according to claim 1, wherein the displaceable moiety comprises an immunoglobulin molecule or an antigen-binding derivative thereof.
3. A method according to claim 1 or 2, wherein the displaceable moiety comprises a bispecific antibody or bispecific antigen-binding antibody derivatives.
4. A method according to any one of claims 1, 2 or 3, wherein the displaceable moiety comprises a portion which facilitates reversible immobilisation on the first surface.
5. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a fusion protein.
6. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a mimotope which is an analogue of the analyte of interest.
7. A method according to any one of the preceding claims, wherein the first surface comprises a plurality of intervening molecules which bind relatively loosely to the displaceable moiety, such that the binding affinity of the intervening moiety for the analyte

of interest is greater than that of the displaceable moiety for the intervening moiety.

8. A method according to claim 8, wherein the intervening molecule is an analogue (e.g. mimotope) of the analyte of interest.

9. A method according to any one of the preceding claims, wherein the capture moiety comprises an immunoglobulin molecule or an antigen-binding variant thereof.

10. A method according to any one of the preceding claims, wherein the displaceable moiety and the capture moiety comprise the members of a specific binding pair.

11. A method according to any one of the preceding claims, wherein the detectable signal comprises the generation of, or the modulation of, an evanescent or acoustic wave.

12. A method according to any one of claims 1-10, wherein capture of the displaceable moiety by the capture moiety directly modulates the electrochemical properties of the capture moiety, which modulation comprises the detectable signal.

13. A method according to any one of the preceding claims, wherein the first and second surfaces are provided on separate respective first and second supports.

14. A method according to any one of claims 1-12, wherein the first and second surfaces are provided on a single support.

15. A method according to any one of the preceding claims, wherein the first and/or second surface is provided on a solid support which is planar, particulate or porous.

16. A method according to any one of the preceding claims, wherein the analyte of interest is selected from the group consisting of: steroid hormones, protein hormones, nucleic acids, peptides, bacterial or viral antigens, and immunoglobulins.

17. Apparatus for performing a method according to any one of the preceding claims, the



apparatus comprising: a first surface having reversibly immobilised thereon a displaceable moiety, wherein the displaceable moiety is displaced from the first surface in the presence of an analyte of interest; and a second surface bearing a capture moiety which specifically binds to the displaceable moiety;

18. Apparatus according to claim 17, further comprising one or more of the following: fluid conducting means; signal detection means for detecting a signal generated by capture of the displaceable moiety by the capture moiety; and data processing means.

19. Apparatus according to claim 17 or 18, comprising filter means provided between the first and second surfaces.

20. A method substantially as hereinbefore described and with reference to the accompanying drawings.

21. Apparatus substantially as hereinbefore described and with reference to the accompanying drawings.

M.H



PATENT COOPERATION TREATY



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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>MJL/C372-02/U</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/GB98/03483</b>	International filing date (day/month/year) <b>23/11/1998</b>	Priority date (day/month/year) <b>21/11/1997</b>
International Patent Classification (IPC) or national classification and IPC <b>G01N33/543</b>		
Applicant <b>UNILEVER PLC et al.</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand  <b>03/05/1999</b>	Date of completion of this report  <b>09.02.00</b>
Name and mailing address of the international preliminary examining authority:   <b>European Patent Office</b> <b>D-80298 Munich</b> <b>Tel. +49 89 2399 - 0 Tx: 523656 epmu d</b> <b>Fax: +49 89 2399 - 4465</b>	Authorized officer  <b>Luis Alves, D</b>  <b>Telephone No. +49 89 2399 8695</b> 

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/03483

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

1-27 as originally filed

### Claims, No.:

1-21 as received on 24/12/1999 with letter of 20/12/1999

### Drawings, sheets:

1/12-12/12 as originally filed

## 2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

## 4. Additional observations, if necessary:

**see separate sheet**

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 20, 21.

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/03483

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
  
- ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. as above are so unclear that no meaningful opinion could be formed (*specify*):  
  
**see separate sheet**
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the said claims Nos. .

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Yes:	Claims	1-16, 19
	No:	Claims	17, 18
Inventive step (IS)	Yes:	Claims	1-16, 19
	No:	Claims	17, 18
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

### 2. Citations and explanations

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB98/03483

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**VI. Certain documents cited**

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/GB98/03483

Reference is made to the following documents cited in the International search report:

D1: WO-A-93/21530

D2: WO-A-97/44664

**Section I:**

Additional observations:

The documents forming the basis of this opinion include sequence listing page 1 as originally filed.

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1. The subject-matter of claims 20 and 21 is totally unclear because no features characterising the claimed methods are defined (Article 6 PCT). Moreover, the wording of said claims is conflicting with the requirements of Rule 6.2(a) PCT. Consequently, no opinion will be established with respect to the novelty, inventive step and industrial applicability of claims 20 and 21 (Article 34(4)(a)(ii) PCT).

**Section V:**

1. D1 discloses a method of determining an analyte in a sample using two surfaces: One onto which an analogue of the analyte is immobilised and another onto which a capture molecule for the analyte is immobilised. The analogue of the analyte is an analyte molecule comprising a marker, such that upon release of the analogue from the first surface and capture on the second surface the marker will generate a detectable signal (see example 6). This example shows determination of an analyte using a first surface coated with a displaceable molecule (marked analyte) which upon application of the sample is captured on the second surface (coated with antibody for the analyte). The fluorescence on the second surface is measured. However, D1 neither discloses nor suggests generation of a signal by the

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/GB98/03483

displaceable molecule only upon capture onto the second surface. The subject-matter of claim 1 and dependent claims 2 to 16 complies with the requirements of Article 33(2) and (3) PCT.

2. D1 discloses surfaces for performing the assay, which may have immobilised thereon antibodies, directly or through spacer molecules (see p.16 third and fourth paragraphs). Thus, the features in claims 17 and 18 are disclosed (Article 33(2) PCT).
3. The feature in claim 19 is neither disclosed nor suggested by any of the cited documents and therefore appears to comply with the requirements of Article 33(2) and (3) PCT.

**Section VI:**

1. Document D2, cited in the International search report as an intermediate document, was published on 27 November 1997, filed on 20 May 1997 and claims a priority date of 23 May 1996. This document may become relevant to the subject-matter of the present claims if the priority date of 21 November 1997 is not validly claimed.

**Section VII:**

1. References to patent application numbers, such as on p.8, line 9, and to internal references, such as on p.13, third paragraph, should be replaced by the corresponding publication numbers.
2. There is a clerical error on claim 8, which refers back to claim 8 instead of claim 7.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/GB98/03483

**Section VIII:**

1. Claim 5 lacks clarity (Article 6 PCT) because the expression "a portion which facilitates reversible immobilisation on the first surface" is vague.
2. The expression "eg mimotope" used in claim 9 introduces a lack of clarity (Article 6 PCT) and should be deleted.



# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>HCM/MJL/GB/C372-02/U</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/GB 98/ 03483</b>	International filing date (day/month/year) <b>23/11/1998</b>	(Earliest) Priority Date (day/month/year) <b>21/11/1997</b>
Applicant <b>UNILEVER PLC et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

### 1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1  
☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

Application No

PCT/EP 98/03483

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 21530 A (MESSERSCHMITT BOELKOW BLOHM ;CAMMANN KARL (DE)) 28 October 1993  see page 19, line 14, paragraph 3 - line 20	1-3,5,8, 10,11, 14-24
Y	see page 23 - page 24; claims 7,9; figure 1; example 6	1,12,13
Y	WO 90 06503 A (ARES SERONO RES & DEV LTD) 14 June 1990 see the whole document	1,12
Y	WO 97 27474 A (YISSUM RES DEV CO ;WILLNER ITAMAR (IL); KATZ EUGENII (IL); COHEN Y) 31 July 1997 see abstract	1,13
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"3" document member of the same patent family

Date of the actual completion of the international search

25 February 1999

Date of mailing of the international search report

08/03/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hart-Davis, J

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/89/03483

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 18867 A (AMERSHAM INT PLC) 29 October 1992 cited in the application see page 2, line 18 - page 3, line 5; examples ---	1-24
A	WO 91 05262 A (UNIV MICHIGAN) 18 April 1991 cited in the application see example 1 ---	1-11, 14-24
P, A	WO 97 44664 A (UNILEVER PLC ; UNILEVER NV (NL)) 27 November 1997 see the whole document ---	1, 7, 9, 21, 22
A	US 5 514 558 A (CERIANI ROBERTO L ET AL) 7 May 1996 see the whole document -----	1, 6

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Application No

PCT/GB 98/03483

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9321530	A	28-10-1993	DE 4216696 A	28-10-1993
WO 9006503	A	14-06-1990	AU 625324 B	09-07-1992
			AU 4755690 A	26-06-1990
			EP 0455642 A	13-11-1991
			JP 5504830 T	22-07-1993
			US 5344784 A	06-09-1994
WO 9727474	A	31-07-1997	AU 1207197 A	20-08-1997
			CA 2244214 A	31-07-1997
			EP 0876602 A	11-11-1998
			NZ 324815 A	23-12-1998
WO 9218867	A	29-10-1992	NONE	
WO 9105262	A	18-04-1991	AU 6753390 A	28-04-1991
			US 5281539 A	25-01-1994
WO 9744664	A	27-11-1997	AU 2961397 A	09-12-1997
US 5514558	A	07-05-1996	US 5532135 A	02-07-1996



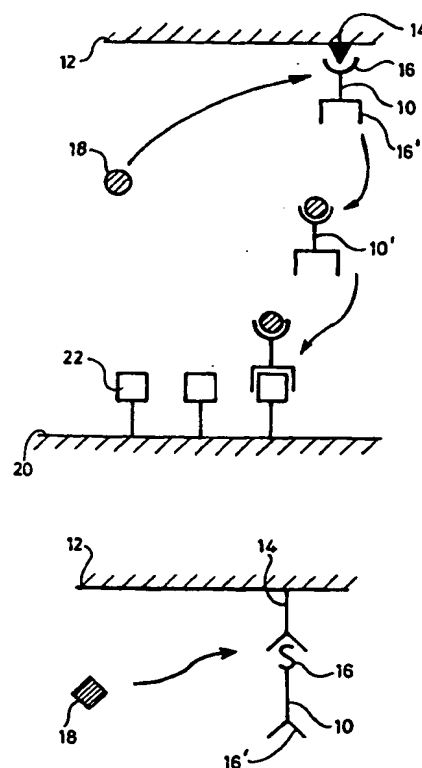
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>G01N 33/543</b>	<b>A1</b>	(11) International Publication Number: <b>WO 99/27368</b> (43) International Publication Date: 3 June 1999 (03.06.99)
<p>(21) International Application Number: PCT/GB98/03483</p> <p>(22) International Filing Date: 23 November 1998 (23.11.98)</p> <p>(30) Priority Data: 97309409.7 21 November 1997 (21.11.97) EP</p> <p>(71) Applicant (for AU BB CA CY GB GD GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).</p> <p>(71) Applicant (for all designated States except AU BB CA CY GB GD GH GM IE IL KE LC LK LS MN MW NZ SD SG SL SZ TT UG ZW): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): BADLEY, Robert, Andrew [GB/GB]; 19 Wellpond Close, Sharnbrook, Bedford MK44 1PL (GB). BERRY, Mark, John [GB/GB]; 2 Fitzwilliam Leys, Higham Ferrers, Northamptonshire NN10 8LY (GB). HOWELL, Stephen [GB/GB]; 10 Heacham Way, Higham Ferrers, Northamptonshire NN10 8LX (GB).</p>	<p>(74) Agent: KEITH W. NASH &amp; CO.; 90-92 Regent Street, Cambridge, CB2 1DP (GB).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> With international search report.</p>	

(54) Title: IMPROVEMENTS IN OR RELATING TO DISPLACEMENT ASSAYS

## (57) Abstract

Disclosed is a method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: reversibly immobilising on a first surface a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal.



Rec'd 22 May 2000  
RECEIVED BY  
ART 34 AMDT

## CLAIMS

1. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having reversibly immobilised thereon a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein said detection is performed by means other than SPR.
2. A method according to claim 1, wherein the detectable signal is generated only when the displaceable moiety is captured by the capture moiety at the second surface.
3. A method according to claim 1 or 2, wherein the displaceable moiety comprises an immunoglobulin molecule or an antigen-binding derivative thereof.
4. A method according to any one of claims 1, 2 or 3, wherein the displaceable moiety comprises a bispecific antibody or bispecific antigen-binding antibody derivatives.
5. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a portion which facilitates reversible immobilisation on the first surface.
6. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a fusion protein.
7. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a mimotope which is an analogue of the analyte of interest.
8. A method according to any one of the preceding claims, wherein the first surface comprises a plurality of intervening molecules which bind relatively loosely to the

displaceable moiety.

9. A method according to claim 8, wherein the intervening molecule is an analogue (e.g. mimotope) of the analyte of interest.
10. A method according to any one of the preceding claims, wherein the capture moiety comprises an immunoglobulin molecule or an antigen-binding variant thereof.
11. A method according to any one of the preceding claims, wherein the displaceable moiety and the capture moiety comprise the members of a specific binding pair.
12. A method according to any one of the preceding claims, wherein the detectable signal comprises the generation of, or the modulation of, an evanescent or acoustic wave.
13. A method according to any one of claims 1-11, wherein capture of the displaceable moiety by the capture moiety directly modulates the electrochemical properties of the capture moiety, which modulation comprises the detectable signal.
14. A method according to any one of the preceding claims, wherein the first and second surfaces are provided on separate respective first and second supports.
15. A method according to any one of claims 1-13, wherein the first and second surfaces are provided on a single support.
16. A method according to any one of the preceding claims, wherein the first and/or second surface is provided on a solid support which is planar, particulate or porous.
17. A method according to any one of the preceding claims, wherein the analyte of interest is selected from the group consisting of: steroid hormones, protein hormones, nucleic acids, peptides, bacterial or viral antigens, and immunoglobulins.
18. Apparatus for performing a method according to any one of the preceding claims, the

apparatus comprising: a first surface having reversibly immobilised thereon a displaceable moiety, wherein the displaceable moiety is displaced from the first surface in the presence of an analyte of interest; and a second surface bearing a capture moiety which specifically binds to the displaceable moiety;

19. Apparatus according to claim 18, further comprising one or more of the following: fluid conducting means; signal detection means for detecting a signal generated by capture of the displaceable moiety by the capture moiety; and data processing means.

20. Apparatus according to claim 18 or 19, comprising filter means provided between the first and second surfaces.

21. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the displaceable moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

22. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface,



said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the intervening moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

23. A method substantially as hereinbefore described and with reference to the accompanying drawings.

24. Apparatus substantially as hereinbefore described and with reference to the accompanying drawings.



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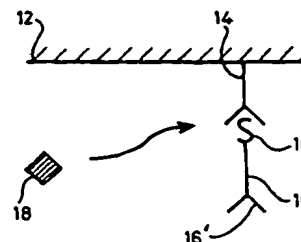
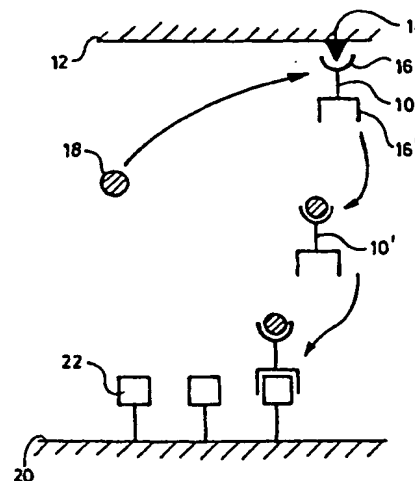
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(57) Abstract

Disclosed is a method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: reversibly immobilising on a first surface a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal.



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## Improvements in or Relating to Displacement Assays

### **Field of the Invention**

This invention relates, *inter alia*, to a method of performing an assay to detect the presence of a substance of interest in a sample, and to apparatus for performing the method.

### **Background of the Invention**

Various assays for detecting the presence and/or amount of a substance of interest in a sample are known. One particular format of assay is the "displacement" assay in which, conventionally, the presence of an analyte of interest in a sample causes the displacement of a labelled substance (either a labelled binding partner or a labelled ligand) from a pre-existing binding partner/ligand complex. Generally speaking the amount of displaced labelled substance will be proportional to the concentration of the analyte of interest. Alternatively, one may employ "competition" assays, in which there is competition between the analyte of interest and a labelled competitor (such as labelled analyte or analogue) for binding to a limiting number of binding sites.

One particular assay is disclosed in WO 91/05262 (University of Michigan), which relates to an assay device, typically comprising a disposable test strip, wherein the presence of an analyte of interest in a test solution with which the test device is contacted, is indicated by a signal (such as a colour) at a distinct location on the device, and the absence of analyte is indicated by the same signal appearing at a different location.

As disclosed in WO91/05262 a first binding means (such as an immobilised antibody) is provided at one location on the test stick, and has binding specificity for the analyte of interest. A "heterobifunctional complex", comprising the analyte covalently coupled to a "signal generating molecule", is reversibly bound to the first binding means. In the presence of free analyte of interest in the sample, heterobifunctional complex is released from the first binding means and transported via "fluid conducting means" (typically a capillary test stick) to a second binding means, having binding specificity for the signal

generating molecule, such that the complex is captured. Typically the signal generating molecule is an enzyme (eg. horseradish peroxidase) such that, when the test stick is dipped into appropriate substrate solution, coloured product (the "signal") forms at the first binding means if no analyte was present in the test sample, or at the second binding means if analyte was present. The signal generating molecule may also be a fluorescent molecule or ultraviolet-absorbing agent.

In this sort of device, there is no interaction between the signal generating molecule and the binding means, beyond simple capture of the complex comprising the signal generating molecule. Thus, the signal generating molecule (the enzyme) is inherently capable of generating a signal (coloured product) whether or not it is released from the first binding means, and the act of capture of the signal generating molecule plays no part in the process of signal generation.

One relatively recent development in relation to assays is the use of devices which employ surface plasmon resonance (SPR). This phenomenon has now been described in several publications and is the basis of evanescent wave biosensors (for a review, see Hutchinson 1995, *Molecular Biotechnology* 3, 47-54, and references therein).

In summary, light incident on an interface between two media of different refractive indices will, at a specific angle of incidence, generate a resonant "evanescent" wave. The resonance is extremely sensitive to changes in the refractive index of the media. A change in the refractive index causes resonance to occur at a new angle of incidence. The change in refractive index is caused by mass binding to a thin (typically gold) film at the interface between the two media: the change in refractive index is proportional to the mass bound to the gold film.

Examples of such devices which utilise the evanescent wave phenomenon include the BIA lite™ and BIA core™ machines sold by Biacore AB (Sweden), the IA Sys™ device sold by Fisons (UK), and the BIOS-1 device sold by Artificial Sensor Instruments (Zurich, Switzerland).

WO 92/18867 discloses an assay for an analyte of interest, the assay involving the displacement of a second reagent from a first reagent immobilised at a displacement surface, said displacement occurring in response to the presence of the analyte of interest, the displaced second reagent then causing a reaction at a detection surface which alters a third reagent immobilised in the detection surface, and wherein the alteration of the third reagent is detected by means of SPR.

WO 92/18867 is primarily concerned with the assay of analytes (such as haptens) of low molecular weight (page 1, lines 4-6) and addresses a lack of sensitivity in assaying such analytes which is apparent in the prior art (page 2, lines 1-17).

Typically, in WO 92/18867 the first reagent is an analogue of the analyte of interest, and the second reagent is an antibody specific for the analyte. Alternatively, the first reagent is an antibody specific for the analyte and the second reagent is a conjugate comprising an analogue (of the low molecular weight analyte) and a high molecular weight protein. The prior art teaches (page 3, lines 6-10) that:

"An analogue of the analyte is a substance which competes with the analyte for binding to a specific binder thereof. Often the analogue structure will be arranged to be as near as possible or even completely identical to the analyte".

Accordingly WO 92/18867 teaches that the analogue and the analyte should be structurally the same as "near as possible", and there is no disclosure or suggestion that there should be a difference in the relative binding affinities between the first reagent, the second reagent, and the analyte of interest.

### **Summary of the Invention**

In a first aspect the invention provides a method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having reversibly immobilised thereon a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced

from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal, wherein said detection is performed by means other than surface plasmon resonance.

The method of the invention may be used to detect the presence of any analyte of interest. Examples of such analytes include antibodies (which may be useful in diagnostic methods), nucleic acids (DNA and/or RNA), hormones (of both the protein and the steroid variety), growth factors and the like. Of particular interest are sex and/or fertility hormones and analogues thereof, such as estrone-3-glucuronide (E3G), progesterone-3-glucuronide (P3G), human chorionic gonadotrophin (hCG), luteinising hormone (LH) and follicle stimulating hormone (FSH), wherein detection of such analytes may be useful in pregnancy testing. Conveniently the analyte is present in a liquid sample, especially a mammalian body fluid, such as blood, serum, urine or saliva.

The method of the invention may be used qualitatively (e.g. to detect the presence of the analyte of interest) or, more preferably, quantitatively such that the amount of analyte present may be determined.

The displaceable moiety and the capture moiety advantageously comprise members of a specific binding pair, such that when the displaceable moiety is displaced from the first surface it may readily be captured specifically at the second surface by the capture moiety. Many specific binding pairs are known to those skilled in the art and include, for example, antigens/antibodies, complementary (i.e. specific base pairing) nucleic acid strands, enzymes/substrates, lectins/sugars, hormones/receptors, and the like.

Generally the first surface will comprise a plurality of the displaceable moieties. Typically the displaceable moieties form a coating or layer attached to the first surface. The displaceable moiety may be directly attached to the first surface. However, more generally, the displaceable moiety will be indirectly attached to the first surface via an intervening molecule, which arrangement facilitates accomplishment of the desired degree of reversible immobilisation of the displaceable moiety.

Typically the intervening molecule will be immobilised to the first surface, by any of a number of conventional chemical techniques, whilst the displaceable moiety is relatively weakly attached to the intervening molecule (and thus reversibly immobilised upon the first surface). Desirably the intervening molecule will be an analogue (such as a mimotope, discussed below) of the analyte of interest, the arrangement being such that the binding affinity of the displaceable moiety (or, in other embodiments, of the intervening molecule) for the intervening molecule will be relatively low (eg. 1-50%, more typically 1-10%, preferably 1-5%) compared to the binding affinity of the displaceable moiety for the analyte of interest. Accordingly, in the presence of the analyte of interest, the analyte will tend specifically to displace the displaceable moiety from the first surface. Advantageously the method of the invention comprises specific, affinity-related displacement of the displaceable moiety, such that the analyte of interest causes quantitative displacement of the displaceable moiety. The relative affinities are desirably selected such that the presence of a small amount of analyte is sufficient to displace a reasonable amount of the displaceable moiety, so that the assay method of the invention is of high sensitivity.

In preferred embodiments the displaceable moiety comprises an immunoglobulin molecule or antigen-binding derivative thereof (such as a Fv, Fab, Fab<sub>2</sub>, scFv, heavy chain variable regions ["Hcv"], such as those obtainable from llamas and camels] and the like). Particularly convenient examples are monoclonal antibodies, bispecific antibodies and "Diabodies", and fusion (chimeric) polypeptides comprising an immunoglobulin molecule or antigen-binding derivative thereof. Fusion proteins may comprise, for example, an immunoglobulin molecule (or antigen-binding derivative thereof) joined to one or more additional functional domains, such as an enzyme (e.g. catalase or glucose oxidase), or a portion which facilitates specific capture by the capture moiety (e.g. a mimotope - i.e. a structural analogue of an epitope recognised by an antibody, or some other molecule such as biotin, avidin or other member of a specific binding pair, wherein the capture moiety comprises the reciprocal member of the specific binding pair), or facilitates reversible immobilisation on the first surface.

In general, the capture moiety will comprise the reciprocal member of a specific binding



pair (i.e. the reciprocal member is that which binds to the member of the pair normally present in the displaceable moiety), and conveniently will be immobilised on the second surface. Conventional chemical methods, well known to those skilled in the art, may be used to effect such immobilisation. It will be understood that the term "immobilisation" in relation to the capture moiety, is intended to indicate that the capture moiety remains bound to the second surface under normal assay conditions, although when an assay has been completed harsh chemical treatment (e.g. very high or very low pH, or the use of chaotropic agents) might be possible to remove the capture moiety from the second surface, such that the surface may be coated with a different capture moiety, enabling performance of the method of the invention for a different analyte of interest. In general however, such treatment is not necessary as one can normally modify the first surface appropriately for use in connection with another analyte.

An important feature of the present invention is the nature of the second surface. In the present invention, the second surface takes what may be described as an "active" part in the signal generation, which contrasts with conventional prior art assays, in which the surface simply acts as in a passive manner as a support. In the present invention, it is the act of capture of the displacement moiety at the second surface which leads indirectly or (preferably) directly to the generation of the detectable signal. Generally the displaceable moiety cannot generate the detectable signal which is actually observed or monitored in the assay unless and until it is captured at the second surface, and generally signal generation depends upon an interaction of the displaceable moiety with the second surface.

The present invention provides a number of advantages over prior art assay methods and devices. Firstly, the invention allows for the use of a generic assay method and device which can readily be modified for use in the detection of any analyte of interest: the same second surface can be used (with the same type of signal generated and detected), only the relatively cheap, simple first surface need be altered, so that the displaceable moiety is displaced in response to the presence of the appropriate analyte. Secondly, the assay method and device, incorporating a specific displacement step, allow for the testing of samples which are heterogeneous (e.g. include particulate matter such as food particles,

blood cells or bacteria) or crude and contain many contaminants (e.g. serum, urine etc.). In addition, the assay of the present invention includes both a specific displacement step, and a specific capture step. This means that the overall specificity of the assay is effectively the mathematical product of the specificities of the displacement and capture steps, giving a far higher specificity than that conferred by most prior art assays. Moreover, this is achievable in a format which, as far as the user is concerned, is essentially a one-step process: the person performing the assay simply has to contact the sample with the first surface, which sets in chain the series of events which lead to the generation of the detectable signal.

In certain embodiments the second surface forms the detection surface of an evanescent or acoustic wave-type biosensor (e.g. as disclosed in EP 0 416 730; EP 0 517 001; or WO 97/04314) such that capture of the displaceable moiety at the surface causes an increase in mass which generates a detectable change in the signal at the second surface, (i.e. capture of the displaceable moiety regulates or modifies a pre-existing signal in a detectable manner).

In some embodiments, capture of the displaceable moiety at the second surface inhibits, in a detectable manner, the generation of a signal, such inhibition constituting the "detectable signal". For example, capture of the displaceable moiety may inhibit a reaction on-going at the second surface: in one embodiment, the second surface comprises a plurality of enzyme molecules which catalyse a reaction producing a detectable (e.g. coloured) product, and the displaceable moiety comprises an immunoglobulin molecule with specific binding activity for the enzyme, such that binding of the displaceable moiety to the enzyme inhibits its catalytic activity (or modifies it in some other way e.g. by binding to an allosteric site), thus producing a detectable change in behaviour of the enzyme.

In alternative preferred embodiments, an evanescent or acoustic wave propagated at the second surface interacts with the displaceable moiety to generate an entirely new signal, rather than the modulation of a pre-existing signal. For example, the displaceable moiety may comprise a fluorophor which is stimulated by the evanescent wave at the second

surface to generate fluorescence: because of the short "range" of the evanescent wave, the fluorophor cannot be stimulated unless the displaceable moiety is captured at the second surface. Further, a degree of specificity is involved in the interaction as the fluorophore and wavelength of the evanescent wave are selected such that the evanescent wave has appropriate characteristics to cause excitation of the fluorophor.

In a preferred embodiment, the capture of the displaceable moiety by the capture moiety modulates an electrochemical property of the capture moiety, which modulation comprises the detectable signal. Suitable methods and substances are disclosed in our co-pending European patent application No. 97309425.3. In particular, the capture moiety typically comprises an immunoglobulin molecule (preferably an antibody) or an antigen-binding derivative thereof, immobilised to an electrically conductive support, wherein binding of the displaceable moiety to the capture moiety alters an electrochemical property of the capture moiety, generating a signal which may be detected by, for example, amperometric, voltametric or coulometric means. Preferably the capture moiety and displaceable moiety are selected such that capture of the displaceable moiety directly alters an electrochemical property (e.g. a redox potential) of the capture moiety.

In some embodiments of the invention, the distance between the first surface and the second surface is very small (e.g. in the range 50 $\mu$ m to 5mm), so that simple diffusion of the displaceable moiety (such as a displaced antibody molecule) between the first and second surfaces is sufficient to provide reasonably rapid response times, where these are required. The distance between the first and second surface may be even smaller than that suggested above in devices which utilise "nanotechnology" principles: in such devices the gap may be as small as the size of a single molecule (e.g. the 5 to 50nm of a typical immunoglobulin molecule or bispecific antibody), in order to minimise the times of mass transport between the first and second surface.

In alternative embodiments, where diffusion of the displaceable moiety from the first to the second surface is not sufficient, it will be necessary to provide some sort of "motive force" to convey the displaceable moiety from the first surface to the second surface. This could be by capillary flow, or by bulk fluid flow (using a pump means) or, for example,

by electrostatic attraction between the displaceable moiety and the second surface.

The first and second surfaces may be provided on separate respective first and second solid supports, which facilitates the inclusion of filter means as described below. Alternatively, the first and second surfaces may be provided on a single solid support. The solid support may be substantially planar, or particulate, and may be porous or impermeable. Where both the first and second surfaces are provided on a single support, the surfaces may be present as distant portions of the support, or may be present as closely adjacent portions of the support (e.g. with one surface surrounded, in two dimensions, by the other surface, or with the two surfaces intimately interdigitated). Examples of particulate supports include beads comprising polymeric substances (e.g. polysaccharides such as dextran; or latex or other material) which are well known to those skilled in the art. Examples of substantially planar supports include the sensor chips used in connection with known evanescent or acoustic wave type biosensors.

It may be preferred in some embodiments to position a filter means between the first and second surface such that particulate materials, present in some samples, may be removed. This may be desirable as such material may have detrimental effects on the assay result (e.g. due to non-specific binding of particulate material at the second surface). Suitable micropore filter membranes are well-known to those skilled in the art.

In a second aspect the invention provides apparatus for performing the method of the first aspect, defined above. Generally the apparatus will comprise: a first surface, having reversibly immobilised thereon a displaceable moiety, wherein the displaceable moiety is displaced from the first surface in the presence of an analyte of interest; and a second surface bearing a capture moiety which specifically binds to the displaceable moiety.

The apparatus defined above may take the form of a relatively cheap, disposable or replaceable component (e.g. as part of a kit) for use in association with other components, such as a "permanent" signal detection means. Alternatively the apparatus of the invention may be provided as substantially a "one piece" device, comprising one or more of the following: fluid conducting means; signal detection means for detecting a signal generated

in the presence of analyte of interest; and data processing means for processing data output from the signal detection means.

The fluid (preferably liquid) conducting means may perform one or more of the following functions: introduction of the fluid sample to the first surface; transport of any displaced moiety from the first surface to the second surface; and removal of waste fluid through the fluid conducting means. Motive means suitable for use with the fluid conducting means may comprise, for example, a rotary or a peristaltic pump.

Desirably the apparatus is adapted for use with analytes of interest which are suspended or dissolved in liquids (especially body fluids, such as blood, serum, saliva and the like).

In a third aspect the invention provides a method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the displaceable moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

In a fourth aspect the invention provides a method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the

displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the intervening moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

With regard to the third and fourth aspects of the invention, it is preferred that the binding affinity of the interaction by which the displaceable moiety is reversibly immobilised on the first surface is only 1-10% (more preferably 1-5%) that of the interaction which tends to cause displacement of the displaceable moiety (i.e. the interaction between the displaceable moiety and the analyte of interest in the third aspect, or the interaction between the intervening moiety and the analyte of interest in the fourth aspect of the invention). The preferred features of the method of the third and fourth aspects of the invention will generally be the same as those of the method of the first aspect, insofar as the methods conform. In the third and fourth aspects of the invention, a preferred means of detecting the signal is by way of SPR, as disclosed in WO92/18867. The invention also provides apparatus for performing the method of the third or fourth aspects defined above.

The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, in which:

Figures 1 - 3, 6 and 10 are schematic illustrations of assay methods in accordance with the invention;

Figures 4, 5A, 5B, 7, and 11 are traces (arbitrary response units against time) showing SPR signal outputs;

Figures 8 and 12 are graphs of SPR response (arbitrary response units) against time (in seconds); and

Figure 9 is a graph (% displaceable moiety captured at second surface, against flow rate in  $\mu\text{l}$  per minute), showing the effect of flow rate on the amount of displaceable moiety captured in one embodiment of the invention, and how at low flow rates 100% of displaceable moiety specifically displaced from the first surface can be captured at the

second surface.

### Example 1

A simple embodiment illustrating the basic concept of the invention is illustrated schematically in Figure 1. In Figure 1, a displaceable moiety is reversibly immobilised at a first surface 12. In the embodiment shown in Figure 1, the displaceable moiety is a bi-specific antibody 10 and the reversible immobilisation is achieved by interaction between the bi-specific antibody 10 and an intervening molecule 14 immobilised, by conventional chemistry, on the first surface 12. For simplicity, only a single molecule 14, and a single specific bispecific antibody 10, are shown. In practice, the first surface 12 would normally be coated with a plurality of intervening molecules 14, and a plurality of bispecific antibody molecules 10 (each having the same binding specificities) would be reversibly immobilised to the surface 12. This reversible immobilisation of the displaceable moiety is such that, under normal assay conditions, and in the absence of the analyte of interest, the displaceable moiety remains attached to the first surface, but is specifically displaced therefrom in the presence of the analyte of interest.

One binding site 16 of the bispecific antibody has binding specificity for the analyte of interest 18. The intervening molecule 14 immobilised on the first surface 12 is a structural analogue of the analyte of interest 18, such that the binding site 16 of the bispecific antibody 10 binds relatively loosely to the molecule 14, thus reversibly immobilising the bispecific antibody 10 on the first surface 12. However, the binding affinity of the binding site 16 for the analogue 14 is much lower (typically 1-10%, preferably 1-5%) than the binding affinity for the analyte of interest 18. Accordingly, upon contacting the first surface 12 with a sample comprising the analyte of interest 18, the analyte 18 competes successfully with analogue 14 for binding to binding site 16, thus specifically displacing the bispecific antibody 10 from the first surface 12. The displaced bispecific antibody 10 is then allowed to contact a second surface 20.

Referring again to Figure 1, the second surface 20 is coated with a plurality of molecules, each of which constitutes a capture moiety 22. The binding site 16 of the bispecific

antibody 10 has binding specificity for the capture moiety 22. Accordingly, the bispecific antibody molecule 10 displaced from the first surface is captured at the second surface 20. This capture generates a detectable signal, which is monitored. In the embodiment illustrated in Figure 1, the second surface 20 forms part of an SPR sensor.

Generally, the signal is generated by an interaction between the captured displaceable moiety 10 and the second (capture) surface 20. For example, the second surface 20 may comprise part of an evanescent wave detection device: capture of the displaceable moiety 10 modifies the characteristics of an evanescent wave propagated at the surface 20, in such a way that a detectable signal is produced. Examples of such devices are those which utilise the technique of surface plasmon resonance (SPR) which are now known in the art, or acoustic wave detection systems, wherein the signal is generated by the increase in mass of material bound to the surface 20.

However, other detection systems may also be envisaged, in which a different sort of interaction occurs between the displaceable moiety (when captured) and the second surface. These include the use of electroactive surfaces, wherein the capture of the displaceable moiety by the capture moiety directly modulates the electrochemical properties of the capture moiety, which modulation comprises the detectable signal. Suitable methods and substances are disclosed in our co-pending application filed on even date herewith, under our reference C319/U.

In the embodiment illustrated in Figure 1, the bispecific antibody 10 remains attached to the analyte of interest 18 when it binds to the capture molecule 22 on the second surface 20. In mass-based detection systems (such as SPR devices) this might be an advantage, especially if the displaceable moiety has a very small mass of its own. However, the continued attachment of the analyte 18 to the displaceable moiety 10 might be disadvantageous in other situations, especially where the generation of the detectable signal is not mass-dependent.



### Example 2

An alternative embodiment (partially illustrated schematically in Figure 2) is also envisaged wherein the nature of the displaceable moiety, and its mode of reversible immobilisation to the first surface, are different to the embodiment illustrated in Figure 1. Functionally comparable components are annotated in Figure 2 using the same reference numerals as in Figure 1.

In Figure 2, there is provided a displaceable moiety 10 reversibly immobilised on a first surface 12, by interaction with an intervening molecule (antibody 14) which is immobilised to the surface 12. Antibody 14 has a specific binding affinity for the analyte of interest 18. The displaceable moiety 10 consists of an antibody molecule fused or covalently coupled to a "mimotope" 16 - a small molecule (normally a peptide, but possibly also a sugar, steroid, nucleic acid or other chemical moiety which may be conjugated to an immunoglobulin) which is a structural analogue of the epitope bound by antibody 14. The mimotope 16 is such that the binding affinity of the antibody 14 for the mimotope 30 is only 1-10% (preferably 1-5%) that of the affinity for the analyte 18. Accordingly, in the presence of the analyte of interest 18, the displaceable moiety 10 is specifically displaced from the first surface 12. The displaceable moiety may then be captured at a second surface (not shown), typically by a capture molecule which recognises the binding site 16' of the antibody portion of the displaceable moiety 10, essentially as indicated in Figure 2. Conveniently the mimotope is a peptide and forms a fusion protein (encoded by a genetic fusion between sequences encoding an immunoglobulin and the peptide). Alternatively the mimotope may be joined to an immunoglobulin molecule (or antigen binding portion thereof) by any one of conventional chemical techniques (see, for example, "Bioconjugate Techniques", Hermanson, Academic Press, 1996).

An advantage of the embodiment illustrated in Figure 2 is that the displaceable moiety 10 when displaced from the first surface 12, consists in large part of a conventional antibody molecule and is not attached to the analyte of interest 18. The presence of the analyte of interest 18 at the second surface (as in the embodiment illustrated in Figure 1) may

sometimes interfere, in some embodiments, with signal generation.

### Example 3

This example relates to the dissociation of human chorionic gonadotrophin (hCG) from an immobilised antibody fragment located at one surface and capture/detection by antibody at a second surface. Figure 3 schematically illustrates this detection principle. This example is performed using the Biacore AB Bialite™ fitted with an upgrade kit and employs the use of surface plasmon resonance.

Initially, a CM5 sensor chip was set up as follows:

- i. A new CM5 sensor chip was docked in the Bialite™ biosensor and equilibrated with HBS (HEPES buffered saline) running buffer. The temperature was maintained at 25°C and the flow rate set to 10  $\mu$ l/min.
- ii. The liquid flow was set to cross two flow cells, flow cell 1 "first surface" and flow cell 2 "second surface" in series. The dextran surface was activated using 1-ethyl(dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) activating chemicals from the Biacore AB amine coupling kit by injecting the EDC/NHS mixture into the sample loop and loading 40  $\mu$ l. The activation was repeated and can be seen marked (a) in figure 4. The Bialite™ response at the first surface is shown as a thin line, whereas the response at the second surface is shown as a thick line. This line thickness identification is used throughout subsequent Bialite™ traces.
- iii. Following activation, 40  $\mu$ l of a 50  $\mu$ g/ml solution of single chain antibody fragment recognising hCG (scFv3299HisH2) was injected across flow cell 1. This was followed by injection of 40  $\mu$ l of a 50 $\mu$ g/ml solution of a whole antibody recognising hCG (3299) across flow cell 2 and a further injection of 40  $\mu$ l of a 50  $\mu$ g/ml solution of scFv3299HisH2 across flow cell 1. These coupling steps can be seen marked (b) in figure 4.

- iv. Unreacted sites on the CM5 sensor chip were blocked by two sequential injections of 40  $\mu$ l of a 1M solution of ethanolamine. These can be seen marked (c) in figure 4.

By way of explanation, the sequence of scFv3299 is as disclosed in WO96/27612 (wherein it is described as FvKC-II), but with the *Bst*EII-*Sac*I DNA linker fragment replaced with a *Bst*EII-*Sac*I fragment encoding a flexible linker yielding the sequence:

FR-4 VH      linker      FR-1 VL

TVTVSSggggsgggsggggsDIELT (seq: ID: No: 1)

The DNA sequence encoding the linker is described by Jackson *et al* ("Selection of variants of antibodies and other protein molecules using display on the surface of bacteriophage fd" in Protein Engineering: A Practical Approach, eds. Rees, Sternberg and Wetzel, publ. IRL Press, Oxford, 1992). It will be appreciated that numerous other anti-hCG antibodies would be equally suitable. Others available are listed in Linscott's Directory of Immunological and Biological Reagents (9th edition, 1996-7) and include, for example, an anti  $\alpha$ -hCG monoclonal available from Biostride Inc. (CA, USA), and an anti  $\beta$ -hCG monoclonal available from Biogenesis Ltd (Poole, Dorset, UK).

The capture/detection of hCG takes place as follows:

- v. 200  $\mu$ l of hCG (10 IU/ml) was incubated with 20  $\mu$ l of a second monoclonal antibody recognising hCG (termed 3468, at 100  $\mu$ g/ml) for 30 minutes at room temperature (again, antibodies other than 3468 would be suitable). This was performed in order to increase the effective mass of hCG and thus increase the sensitivity of detection.
- vi. 40  $\mu$ l of the premixed hCG-3468 solution was injected across flow cell 1. This can be seen marked (a) in figure 5A.
- vii. Immediately following the injection, the buffer flow was altered to pass over the second surface after leaving the first surface. The point at which this took place is marked (b) in figure 5B.

Figure 5A shows that hCG-3468 complex dissociating from the first surface can be bound and detected at the second surface by capture with anti-hCG antibody 3299 (Fig. 5B). Those skilled in the art will appreciate that, in practice, there is no need for the first surface to generate a detectable signal - such was the arrangement in this example for the purposes of investigation only. In a normal arrangement only the capture (second) surface would give rise to a detectable signal, such that the first surface could be any suitable solid support. Moreover, in the assay method of the invention, conditions would be arranged such that hCG was displaced from surface 1 only in the presence of the analyte of interest. The example nevertheless demonstrates the principle of the invention.

#### **Example 4**

This example relates to the specific displacement of an antibody (4155) recognising estrone 3-glucuronide (E3G) from a low affinity E3G analogue (estradiol 3-glucuronide [ED3G]) at one surface and capture and detection by an antibody recognising immunoglobulin at a second surface. Figure 6 schematically illustrates this detection principle.

The 4155 monoclonal cell line was prepared and screened according to the methods described by Gani *et al.*, (1994 J. Steroid Biochem. Molec. Biol. 48, 277-282). The Gani *et al.* publication relates to development of anti-progesterone antibodies, but essentially identical techniques were employed in producing antibodies reacting with estrone and analogues thereof. Antibodies other than that obtainable from cell line 4155 may readily be produced by those skilled in the art (using such techniques): such antibodies would have qualitatively similar properties. Moreover, a commercially available anti-estrone glucuronide monoclonal antibody (from Wallaceville Animal Research Centre, New Zealand) is described in Linscott's Directory of Immunological and Biological Reagents (9th edition, 1996-7).

Initially, a CM5 sensor chip was set up as follows:

- i. A new CM5 sensor chip was docked in the Bialite™ biosensor and equilibrated with HBS running buffer. The temperature was maintained at 25°C and the flow rate set to 10 µl/min.

- ii. The liquid flow was set to cross a first surface ("surface 1") and a second surface ("surface 2") in series. The dextran surface was activated using EDC and NHS activating chemicals from the Biacore AB amine coupling kit by injecting the EDC/NHS mixture into the sample loop and loading 40  $\mu$ l. This can be seen marked (a) on figure 7.
- iii. The buffer flow was set to cross only surface 2 and 40  $\mu$ l of rabbit anti-mouse IgG in sodium acetate buffer, pH 5.0 was injected. This can be seen marked (b) on figure 7.
- iv. Following IgG coupling to surface 2 the buffer flow was changed to flow only over surface 1 and 40  $\mu$ l ethylene diamine (EDA) (20% v/v) injected. This can be seen marked (c) on figure 7.
- v. 20  $\mu$ l of ED3G (5 mg/ml) was added to 80  $\mu$ l of a solution of NHS/EDC activation solution, mixed and incubated for 7 minutes at room temperature. 40  $\mu$ l of this preincubated ED3G solution was then injected across surface 1 (this can be seen marked (d) on figure 7).
- vi. The buffer flow was set to flow only over surface 2 and 40  $\mu$ l of a 1M solution of ethanolamine was injected. This can be seen marked (e) on figure 7.

Displacement and recapture of 4155 was performed as follows:

- vii. 4155 was loaded onto surface 1 by the injection of 20  $\mu$ l of a 100  $\mu$ g/ml solution of 4155.
- viii. Specific displacement from surface 1 and recapture/detection at surface 2 was induced by the injection of a 1 mg/ml solution of E3G across surfaces 1 and 2 for two minutes at various flow rates. The results of this can be seen in figure 8, in which the filled boxes show the specific displacement of 4155 from surface 1 by E3G and the open boxes show the capture of 4155 at surface 2. Increasing box

size denotes an increase in flow rate where the smallest size box represents 2  $\mu\text{l}/\text{min}$  and subsequent box sizes are of data collected at 5, 10 and 20  $\mu\text{l}/\text{min}$ .

The percentage of the displaced 4155 captured and detected at surface 2 is clearly related to the flow rate (see figure 9). An increase in flow rate decreases the percentage of displaced material. Low flow rates (less than 5  $\mu\text{l}/\text{min}$ ) can ensure all the displaced material is captured.

This example shows how analyte (E3G) can be assayed by inducing the specific displacement of a molecule reversibly bound to one surface and subsequent recapture of the displaced material at a second distinct surface by, for example, an antibody recognising the displaced molecule.

#### **Example 5**

This example relates to the specific displacement of an antibody (4101) recognising estrone 3-glucuronide (E3G) from a low affinity E3G analogue (estradiol 3-glucuronide [ED3G]) at one surface and capture by E3G at a second, detecting surface. Figure 10 schematically illustrates this detection principle.

Initially, a CM5 sensor chip was set up as follows:

- i. A new CM5 sensor chip was docked in the Bialite™ biosensor and equilibrated with HBS running buffer. The temperature was maintained at 25°C and the flow rate set to 10  $\mu\text{l}/\text{min}$ .
- ii. The liquid flow was set to cross a first surface ("surface 1") and a second surface ("surface 2") in series. The dextran surface was activated using EDC and NHS activating chemicals from the Biacore AB amine coupling kit by injecting the EDC/NHS mixture into the sample loop and loading 40  $\mu\text{l}$ . This can be seen marked (a) on figure 11.
- iii. 40  $\mu\text{l}$  of EDA (20% v/v) was injected across surface 1 and 2. This can be seen

marked (b) on figure 11.

- iv. 20  $\mu$ l of ED3G (5 mg/ml) was added to 80  $\mu$ l of a solution of NHS/EDC activation solution, mixed and incubated for 7 minutes at room temperature. 40  $\mu$ l of this preincubated ED3G solution was then injected across surface 1 (this can be seen marked (c) on figure 11).
- v. 20  $\mu$ l of E3G (5 mg/ml) was added to 80  $\mu$ l of a solution of NHS/EDC activation solution, mixed and incubated for 7 minutes at room temperature. 40  $\mu$ l of this preincubated E3G solution was then injected across surface 2 (this can be seen marked (d) on figure 11).

Specific displacement and capture of 4101 was performed as follows:

- vi. 4101 was loaded onto surface 1 by the injection of 20  $\mu$ l of a 100  $\mu$ g/ml solution of 4101 across surface 1.
- vii. Displacement of 4101 from surface 1 and recapture/detection at surface 2 was induced by the injection of 20  $\mu$ l of a 1 mg/ml solution of estrone 3-sulphate [E3S] across surface 1 and 2. The displacement from surface 1 and capture/detection at surface 2 can be seen in figure 12.

In this example E3S represents the analyte. This example shows how analyte can be assayed by inducing the displacement of a molecule reversibly bound to a lower affinity analogue of the analyte (ED3G) at one surface and subsequently recaptured and detected at a second distinct surface by binding to a higher affinity analogue of the analyte (E3G).

#### **Example 6**

This example shows the production of a colour change brought about by the displacement/capture of a glucose, oxidase/antibody conjugate in response to analyte addition. The displaced glucose oxidase/antibody conjugate is captured on a thin layer of

starch impregnated with KI, horse radish peroxidase and a capture molecule. The captured glucose oxidase/antibody conjugate utilises glucose to generate  $H_2O_2$ , which oxidises iodide to iodine in the presence of peroxidase. The iodine generated then reacts with starch, resulting in a colour change from brown to blue.

Two surfaces are set up as follows:

**First Surface ("Surface 1")**

- i. An estradiol 3-glucuronide (ED3G)/ovalbumin conjugate is prepared by resuspending 2.6 mg ED3G in 2 ml of a freshly prepared solution of EDC (0.1 M) and NHS (0.02 M) and incubating for 15 min at room temperature.
- ii. To the ED3G solution, 2 ml ovalbumin (10 mg/ml) is added and this is incubated for 2.5 h at room temperature with constant mixing.
- iii. The conjugated ED3G/ovalbumin solution is then dialysed for 16 h against 1L phosphate buffered saline containing 0.1% sodium azide ("PBSA").
- iv. Griener HB wells are coated with the dialysed ED3G-ovalbumin conjugate by incubating 100  $\mu$ l ED3G-ovalbumin (diluted 1 in 10 with PBSA) in the wells for 2 h at room temperature.
- v. A conjugate of glucose oxidase and monoclonal antibody 4155 is prepared by mixing 1ml glucose oxidase in 100 mM phosphate buffer pH 6.5 with 1 ml of monoclonal antibody 4155 (also in 100 mM phosphate buffer pH 6.5) in a glass tube at a protein concentration of 5 mg/ml. To this 220  $\mu$ l of 0.02% glutaraldehyde is added dropwise with constant mixing. The tube is wrapped in aluminium foil and mixed end over end for 30 min at room temperature. 250  $\mu$ l of 1 M ethanolamine is added and mixing continued for a further 30 min. The solution is then dialysed against 100 mM PBS at room temperature for 24 h then centrifuged at 10,000 rpm for 30 min at 4°C.



- vi. The Griener HB surface is then primed by incubating 100  $\mu$ l glucose oxidase-4155 conjugate per well for 1 h at room temperature. Wells are then rinsed with PBS to remove unbound glucose oxidase/4155 conjugate. Wells are stored containing 100  $\mu$ l PBS at 4°C until required.

#### **Second Surface ("Surface 2")**

- i. A 200  $\mu$ l solution containing 100  $\mu$ l estrone 3-glucuronide (E3G)-ovalbumin conjugate (prepared as described for the ED3G-ovalbumin conjugate), 5% (w/v) starch, 10 mg/ml horseradish peroxidase and 0.1 M KI in PBS is incubated in a Griener HB well for 16 h at room temperature. The surface is brown in colour.

Analyte (1 ml estrone 3 glucuronide at 0.1 mM) in a 10 mM glucose solution in PBS is pumped at 0.2 ml/min into the well with the primed surface 1 and at the same time solution from this well is pumped at 0.2 ml/min into the well containing surface 2. In order that the well with surface 2 does not overflow, solution is also pumped out of the well at 0.2 ml/min.

Upon starting the flow of test solution from surface 1 to surface 2 the colour of surface 2 changes from brown to blue as the iodide is oxidised to iodine by the  $H_2O_2$  generated by the oxidation of glucose by glucose oxidase.

#### **Example 7**

This example relates to the modulation of fluorescence intensity at a surface in response to analyte. Upon challenge with analyte, molecules are displaced from a first surface. These displaced molecules are then able to modulate the intensity of fluorescence generated at a second surface.

Two surfaces are set up as follows:

#### **First Surface ("Surface 1")**

- i. An estradiol 3-glucuronide (ED3G)/ovalbumin conjugate is prepared by resuspending 2.6 mg ED3G in 2 ml of a freshly prepared solution of EDC (0.1 M) and NHS (0.02 M) and incubating for 15 min at room temperature.
- ii. To the ED3G solution 2 ml ovalbumin (10 mg/ml) is added and this is incubated for 2.5 h at room temperature with constant mixing.
- iii. The conjugated ED3G/ovalbumin solution is then dialysed for 16 h against 1L PBSA.
- iv. Griener HB wells are coated with the dialysed ED3G/ovalbumin conjugate by incubating 100  $\mu$ l ED3G/ovalbumin (diluted 1 in 10 with PBSA) in the wells for 2 h at room temperature.
- v. A conjugate of cellulose binding domain (CBD)/monoclonal antibody 4155 is prepared by mixing 1 ml CBD in 100 mM phosphate buffer pH 6.5 with 1 ml of monoclonal antibody 4155 (also in 100 mM phosphate buffer pH 6.5) in a glass tube at a protein concentration of 5 mg/ml. To this 220  $\mu$ l of 0.02% glutaraldehyde is added dropwise with constant mixing. The tube is wrapped in aluminium foil and mixed end over end for 30 min at room temperature. 250  $\mu$ l of 1 M ethanolamine is added mixing continued for a further 30 min. The solution is then dialysed against 100 mM PBS at room temperature for 24 h then centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant is stored at 4°C until required.
- vi. The Griener HB surface is then primed by incubating 100  $\mu$ l CBD-4155 conjugate per well for 1 h at room temperature. Wells are then rinsed with PBSA to remove unbound CBD/4155 conjugate.

#### **Second Surface ("Surface 2")**

- i. A 1 ml solution of H<sub>3</sub>PO<sub>4</sub>swollen Avicel (0.2%) is incubated with 50  $\mu$ l locust bean gum (LBG)-FITC conjugate for 1h with constant mixing at room temperature.

- ii. LBG-FITC Avicel is washed by subjecting the particles to centrifugation at 1300 rpm for 10 min at room temperature and resuspending the pellet in PBS. This wash procedure is repeated a second time.
- iii. Following the second wash the LBG-FITC loaded particles are resuspended in 50  $\mu$ l PBS and stored at 4°C until required.

Analyte (100  $\mu$ l E3G at 1 mg/ml) is incubated for 1 h in primed Griener HB wells (surface 1) at room temperature. The incubated solution is then removed from the wells and added to LBG-FITC loaded particles. A decrease in fluorescence intensity due to the displacement of LBG-FITC by CDB-4155 on the Avicel particles is monitored using a fluorescent activated cell sorter, or by a cell sorter (e.g. the Coulter Elite™ cell sorter).

### Example 8

This example shows the production of an electrical change brought about by the displacement/capture of a bispecific antibody fragment construct in response to analyte addition. It relates to the specific displacement of the first antibody binding site (recognising estrone-3-glucuronide (E3G) and derived from mouse monoclonal antibody 4155) from a low affinity E3G analogue (estradiol 3-glucuronide (ED3G)) from a first surface, and capture and detection through the second antibody binding site recognising an electroactive molecule immobilised at a second surface. The second surface is a gold electrode coated with a monolayer of the electroactive molecule. The electroactive molecule is a carbazole residue, coupled to a hexyl pendant portion, which is in turn coupled to the gold surface by a thiol group. Our co-pending European Patent application, filed on even date herewith, describes how the binding of selected antibodies can alter (modulate) the electrical current flowing through the electrode under the influence of an applied voltage. Here the binding of the bispecific antibody fragment to the electroactive molecule thus modulated the current flow in a manner which was directly related to the concentration of analyte originally introduced.

The 4155 monoclonal cell line was prepared and screened according to methods described by Gani *et al*, (1994 J. Steroid Biochem. Molec. Biol. **48**, 277-282). The Gani *et al* publication relates to the development of anti-progesterone antibodies, but essentially identical techniques were employed in producing antibodies reacting with estrone and analogues thereof. Antibodies other than that obtainable from cell line 4155 may readily be produced by those skilled in the art (using such techniques): such antibodies would have qualitatively similar properties. Monoclonal antibodies against the electroactive carbazole molecule, another hapten, can also be produced using these methods. The cloning of the genes for the two antibody binding specificities and their use in the construction of a bispecific antibody fragment can easily be accomplished by methods well known to those skilled in the art; (see for example WO 97/14719).

Two surfaces are set up as follows:

**First surface ("Surface 1")**

- i. An estradiol 3-glucuronide (ED3G)/ovalbumin conjugate is prepared by resuspending 2.6mg of ED3G in 2ml freshly prepared solution of EDC (0.1M) and NHS (0.02M) and incubating for 15 mins at room temperature.
- ii. To the ED3G solution, 2ml ovalbumin (10mg/ml) is added and this is incubated for 2.5hr at room temperature with constant mixing.
- iii. The conjugated ED3G/ovalbumin solution is then dialysed for 16hr against 1L phosphate buffered saline containing 0.1 % sodium azide ("PBSA").
- iv. Greiner HB wells are coated with dialysed ED3G-ovalbumin conjugate by incubating 100 $\mu$ l ED3G-ovalbumin (diluted 1 in 10 with PBSA) in the wells for 2 hr at room temperature.
- v. The Greiner HB-ED3G-ovalbumin surface is then primed by incubating 100 $\mu$ l of a solution of the bispecific antibody fragment (100 $\mu$ g/ml in PBSA) per well for 1 hr at room

temperature. Wells are then rinsed with PBSA to remove unbound bispecific antibody fragment. Wells were stored containing 100 $\mu$ l of PBSA at 4°C until required.

#### Second surface ("Surface 2")

- i. The thiol terminus alkyl pendant electroactive (carbazole) molecule was dissolved in nitrogen purged ethanol or DMF solution.
- ii. A clean pure gold surface was dipped into the solution containing the electroactive moiety for 24hr in the dark.
- iii. The electroactive monolayer on the gold surface was washed in a suitable solvent (e.g. dichloromethane) to remove any unbound molecules and then stored overnight in a suitable solvent that has been nitrogen purged.
- iv. The electrode was dried in a stream of nitrogen to remove solvent from the electrode surface.
- v. The electrode was transferred through a series of increasingly dilute acetonitrile: water mixes to improve the monolayer's molecular orientation, which results in a monolayer which is stable in an aqueous environment.

#### Assay

Electrochemical measurements are performed by cyclic voltammetry or chronoamperometry on a Princeton Applied Research Corporation Scanning Potentiostat (model 362) together with a Bryan flat bed X-Y recorder (model A25000) and a EH&G model 273A Princeton Applied Research Potentiostat/Galvanostat (using Echem and Lotus 123 to process the data).

- (a) The background electrochemistry is read and stored once a stable scan had been produced.

- (b) The sample to be read is added to surface 1 (the well) and incubated for 15 minutes at room temperature.
- (c) The sample is removed from the well and added to surface 2 (the electrode surface), and again incubated at room temperature for 15 minutes.
- (d) The electrode surface is washed with MilliQ water and the electrochemistry read using the same conditions as (b) above. The modulation of the current flow gives a measure of the analyte concentration which may be expressed in molar concentration units if the system has been calibrated against standard E3G samples.

## CLAIMS

1. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having reversibly immobilised thereon a displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein said detection is performed by means other than SPR.
2. A method according to claim 1, wherein the detectable signal is generated only when the displaceable moiety is captured by the capture moiety at the second surface.
3. A method according to claim 1 or 2, wherein the displaceable moiety comprises an immunoglobulin molecule or an antigen-binding derivative thereof.
4. A method according to any one of claims 1, 2 or 3, wherein the displaceable moiety comprises a bispecific antibody or bispecific antigen-binding antibody derivatives.
5. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a portion which facilitates reversible immobilisation on the first surface.
6. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a fusion protein.
7. A method according to any one of the preceding claims, wherein the displaceable moiety comprises a mimotope which is an analogue of the analyte of interest.
8. A method according to any one of the preceding claims, wherein the first surface comprises a plurality of intervening molecules which bind relatively loosely to the

displaceable moiety.

9. A method according to claim 8, wherein the intervening molecule is an analogue (e.g. mimotope) of the analyte of interest.
10. A method according to any one of the preceding claims, wherein the capture moiety comprises an immunoglobulin molecule or an antigen-binding variant thereof.
11. A method according to any one of the preceding claims, wherein the displaceable moiety and the capture moiety comprise the members of a specific binding pair.
12. A method according to any one of the preceding claims, wherein the detectable signal comprises the generation of, or the modulation of, an evanescent or acoustic wave.
13. A method according to any one of claims 1-11, wherein capture of the displaceable moiety by the capture moiety directly modulates the electrochemical properties of the capture moiety, which modulation comprises the detectable signal.
14. A method according to any one of the preceding claims, wherein the first and second surfaces are provided on separate respective first and second supports.
15. A method according to any one of claims 1-13, wherein the first and second surfaces are provided on a single support.
16. A method according to any one of the preceding claims, wherein the first and/or second surface is provided on a solid support which is planar, particulate or porous.
17. A method according to any one of the preceding claims, wherein the analyte of interest is selected from the group consisting of: steroid hormones, protein hormones, nucleic acids, peptides, bacterial or viral antigens, and immunoglobulins.
18. Apparatus for performing a method according to any one of the preceding claims, the



apparatus comprising: a first surface having reversibly immobilised thereon a displaceable moiety, wherein the displaceable moiety is displaced from the first surface in the presence of an analyte of interest; and a second surface bearing a capture moiety which specifically binds to the displaceable moiety;

19. Apparatus according to claim 18, further comprising one or more of the following: fluid conducting means; signal detection means for detecting a signal generated by capture of the displaceable moiety by the capture moiety; and data processing means.

20. Apparatus according to claim 18 or 19, comprising filter means provided between the first and second surfaces.

21. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface, said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the displaceable moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

22. A method of detecting the presence of an analyte of interest in the sample, the method comprising the steps of: providing a first surface having a displaceable moiety reversibly immobilised thereon by means of an interaction with an intervening moiety between the first surface and the displaceable moiety; exposing the first surface to a sample comprising the analyte of interest, the analyte of interest specifically displacing the displaceable moiety from the first surface; causing the displaceable moiety displaced from the first surface to contact a second surface bearing a capture moiety which specifically binds to the displaceable moiety, so as to capture the displaceable moiety on the second surface,

said capture generating a detectable signal; and detecting the signal; wherein the binding affinity of the intervening moiety for the analyte of interest is greater than that of the displaceable moiety for the intervening moiety.

23. A method substantially as hereinbefore described and with reference to the accompanying drawings.

24. Apparatus substantially as hereinbefore described and with reference to the accompanying drawings.

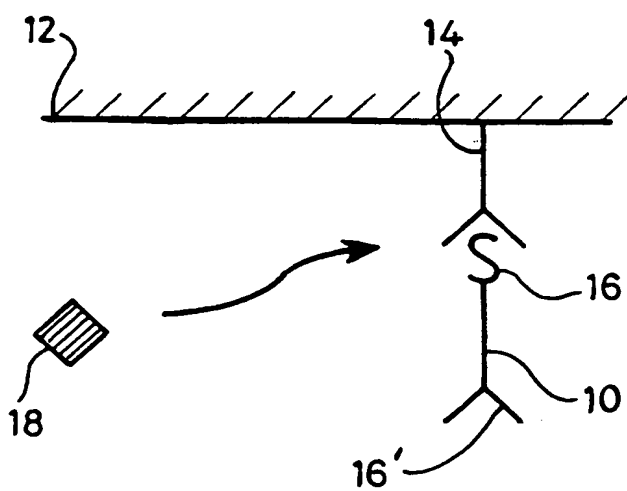
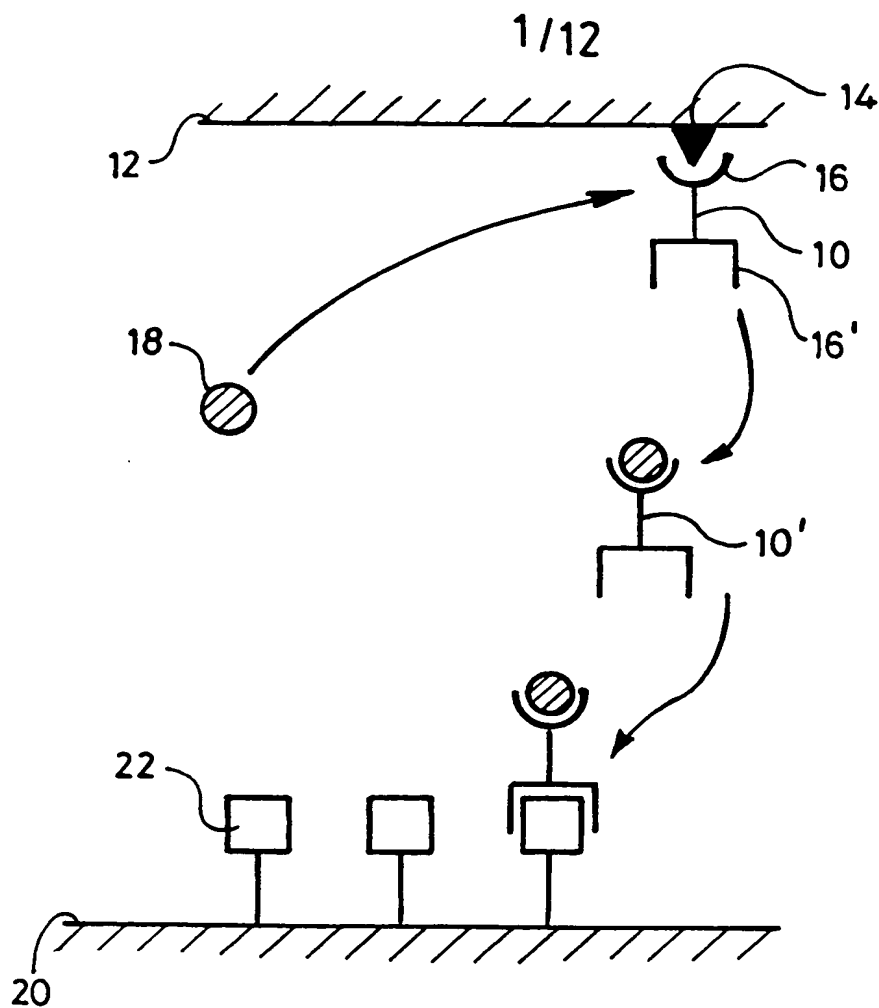


Fig. 1

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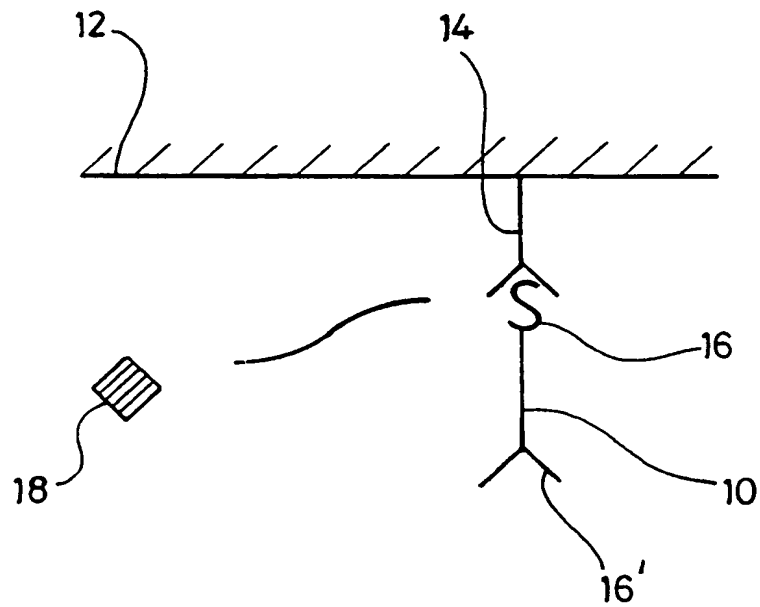
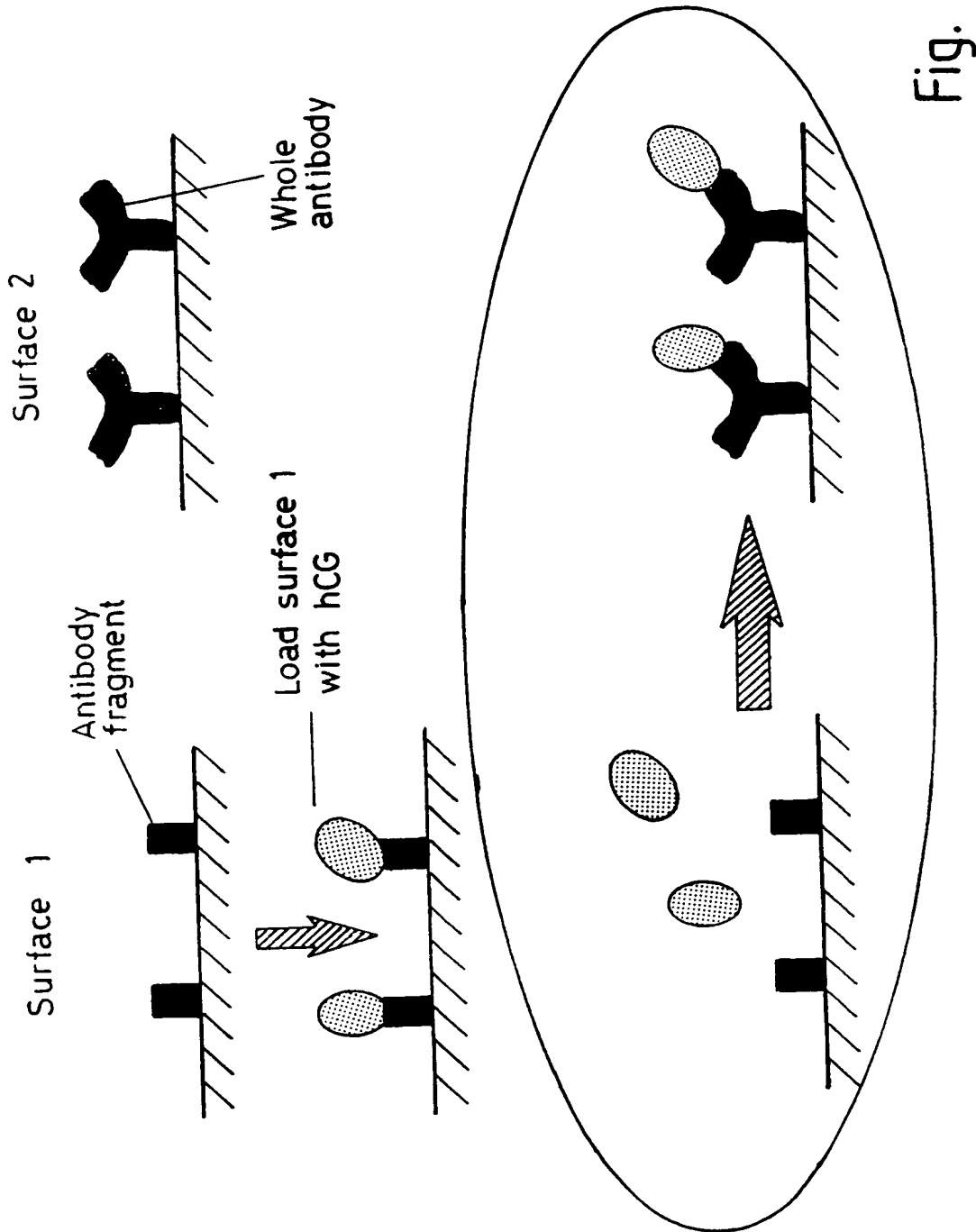


Fig. 2

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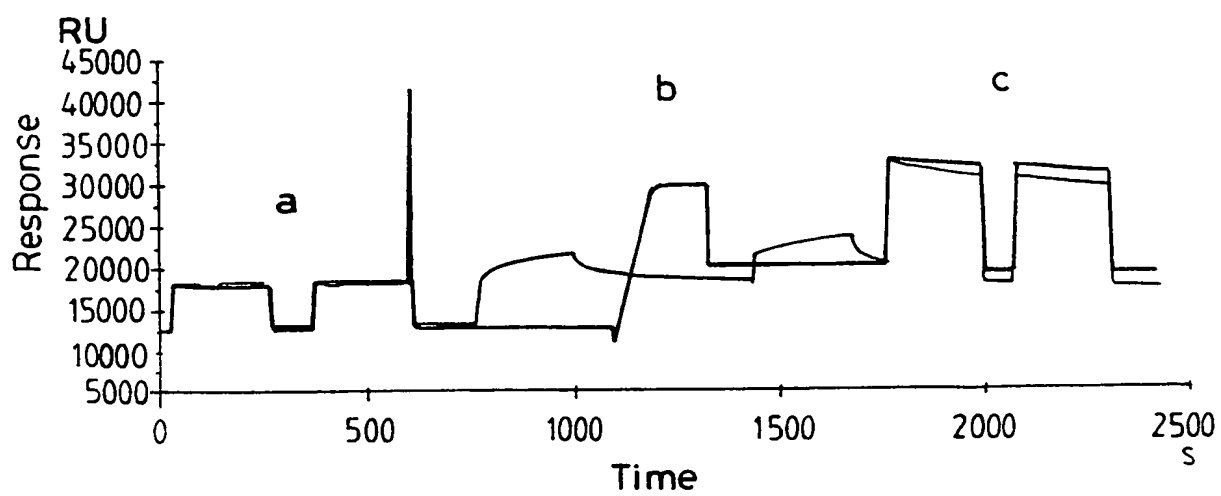


Fig. 4

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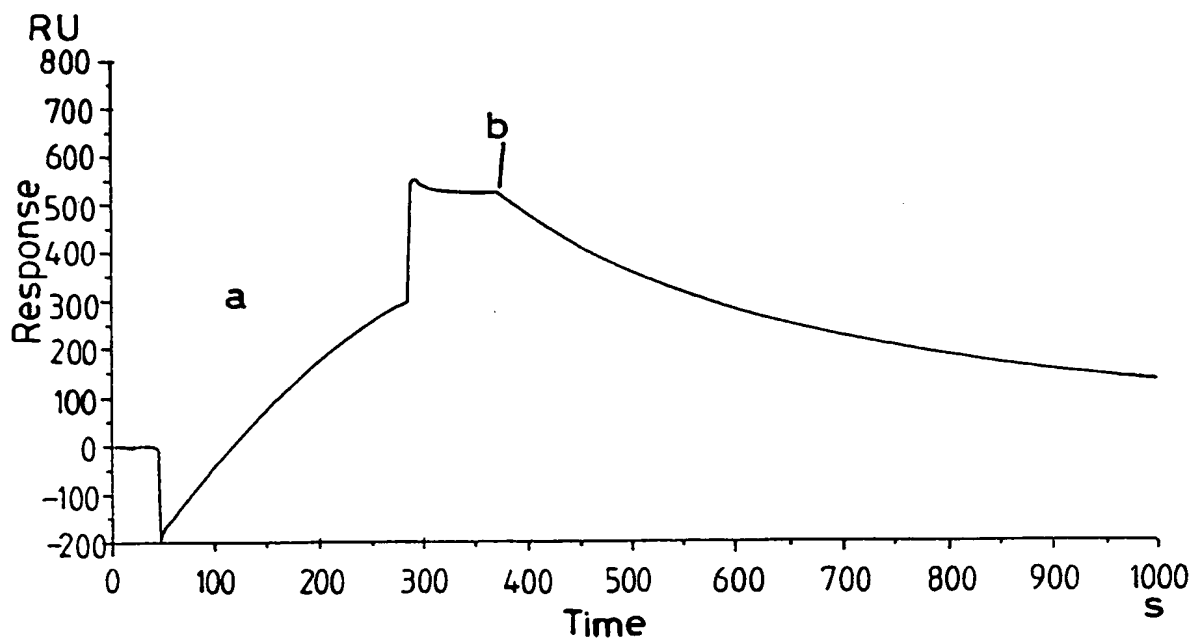


Fig. 5A

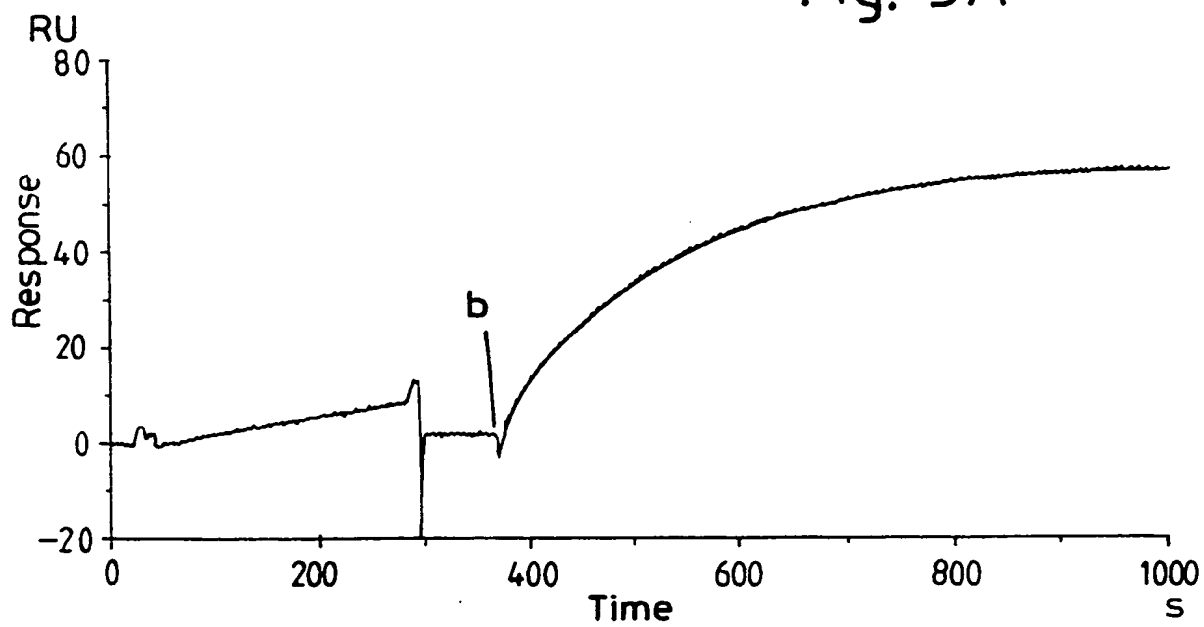


Fig. 5B

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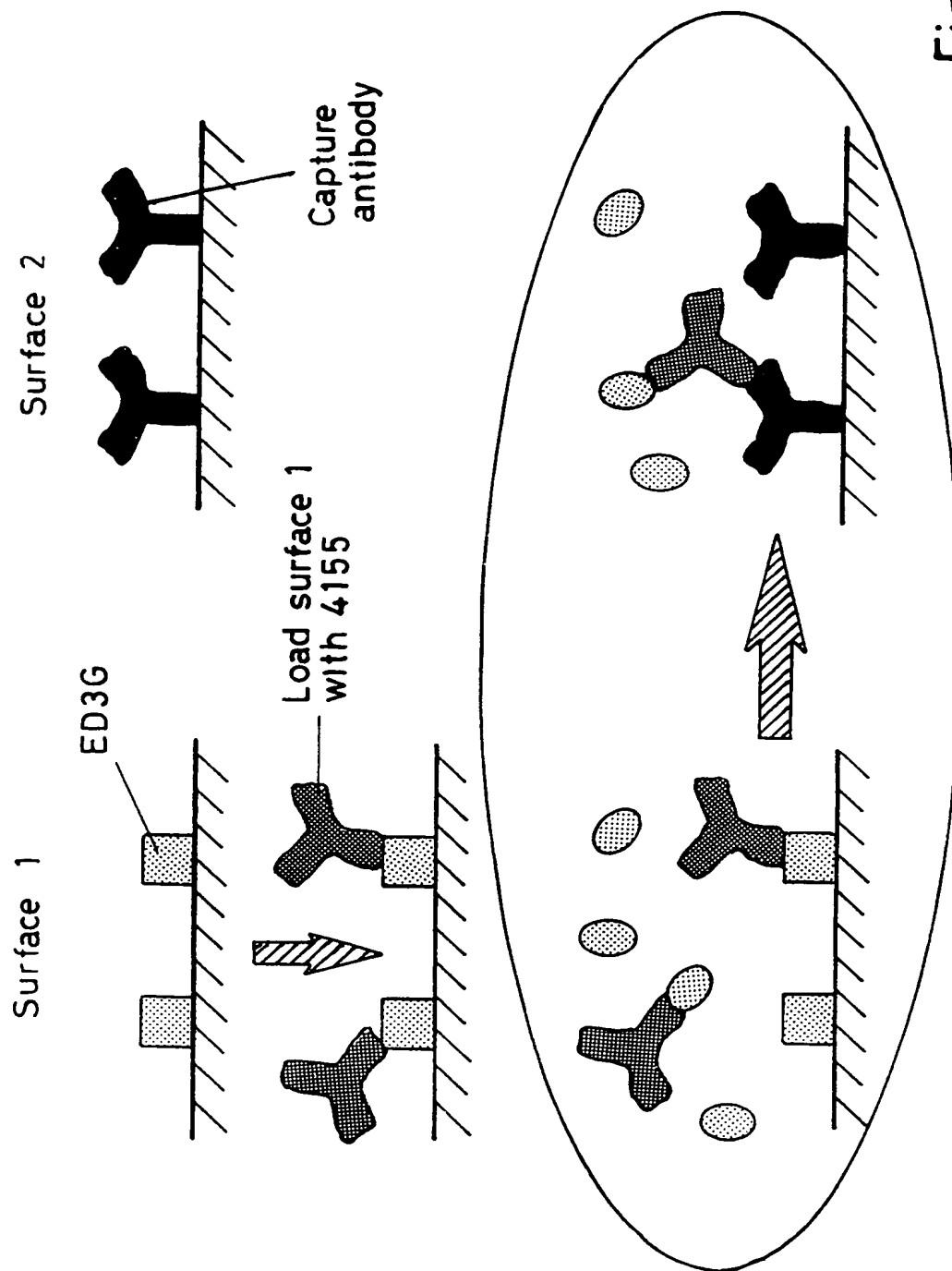


Fig. 6



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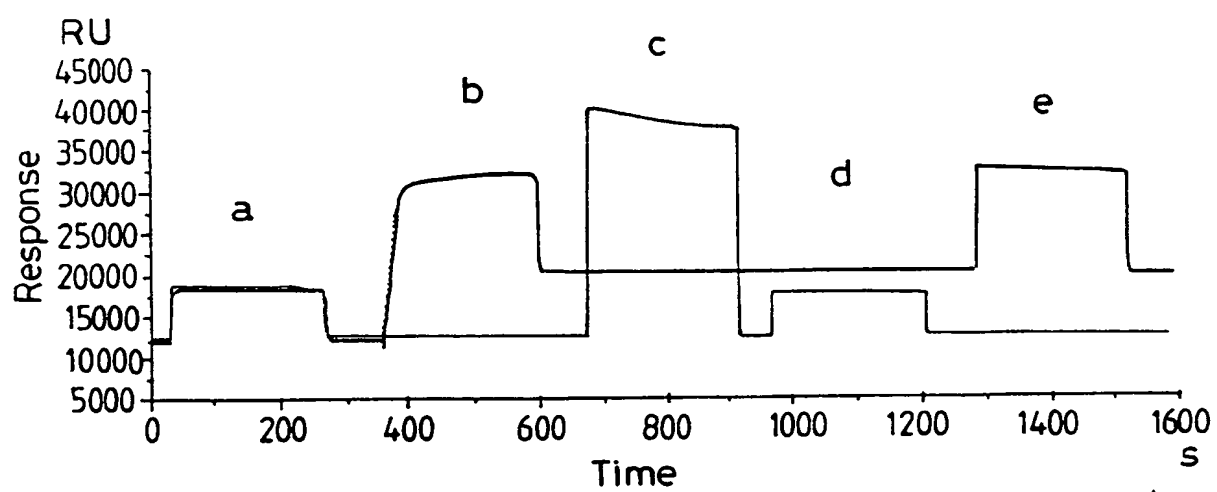


Fig. 7

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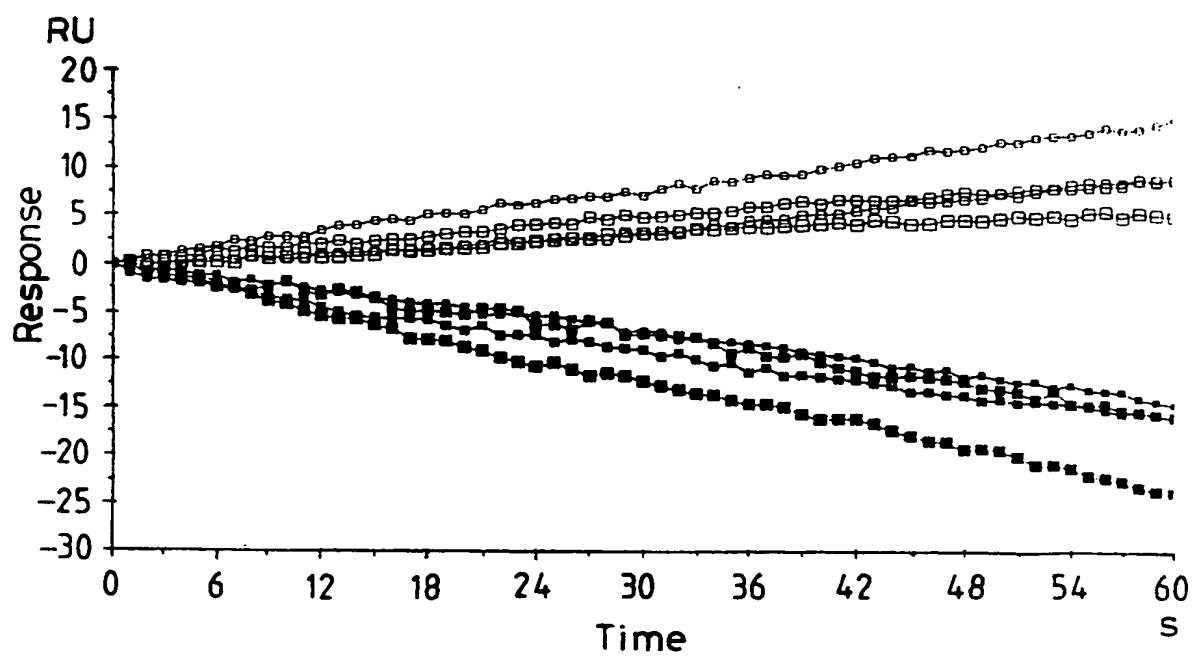


Fig. 8

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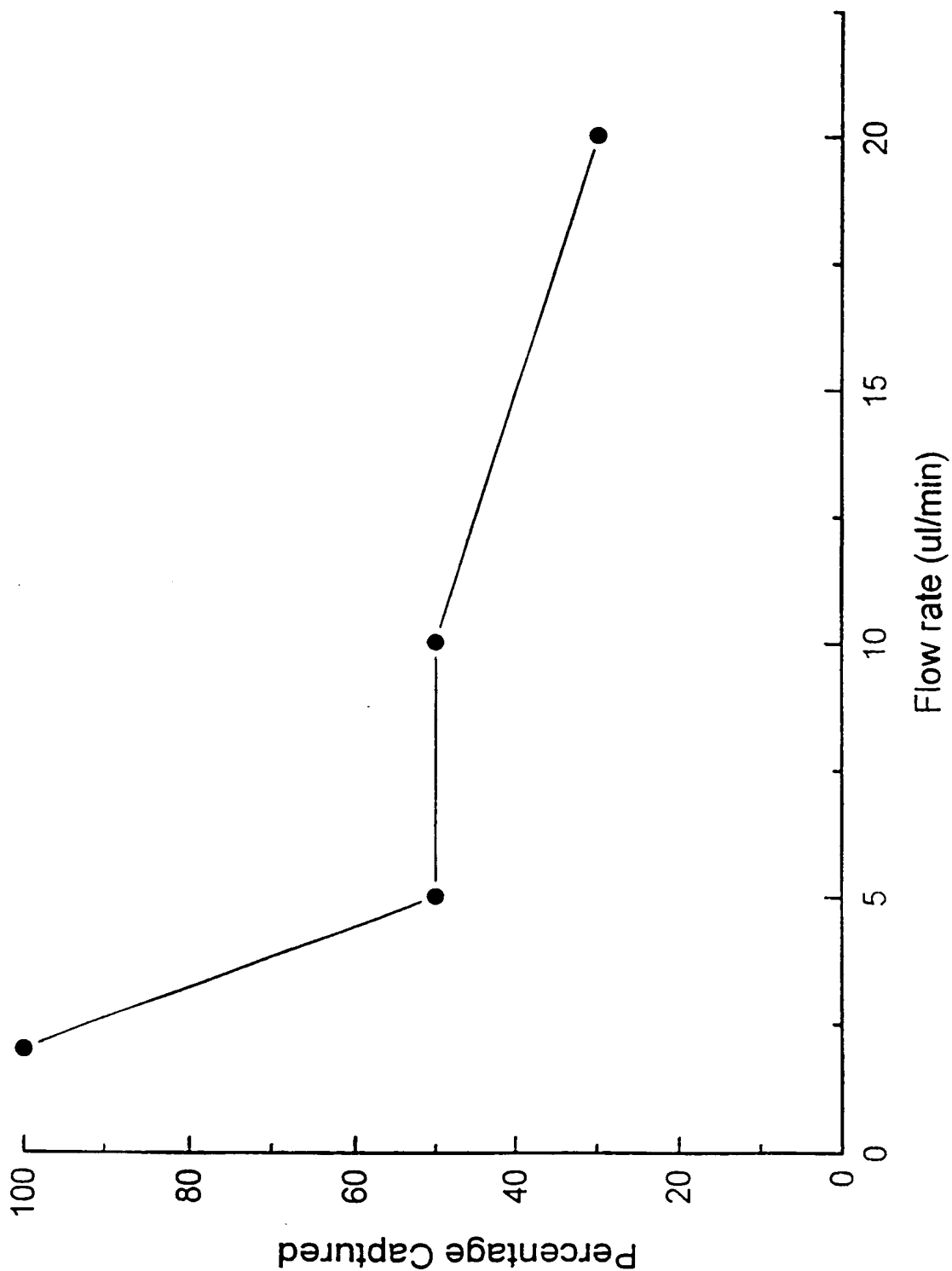


Fig. 9

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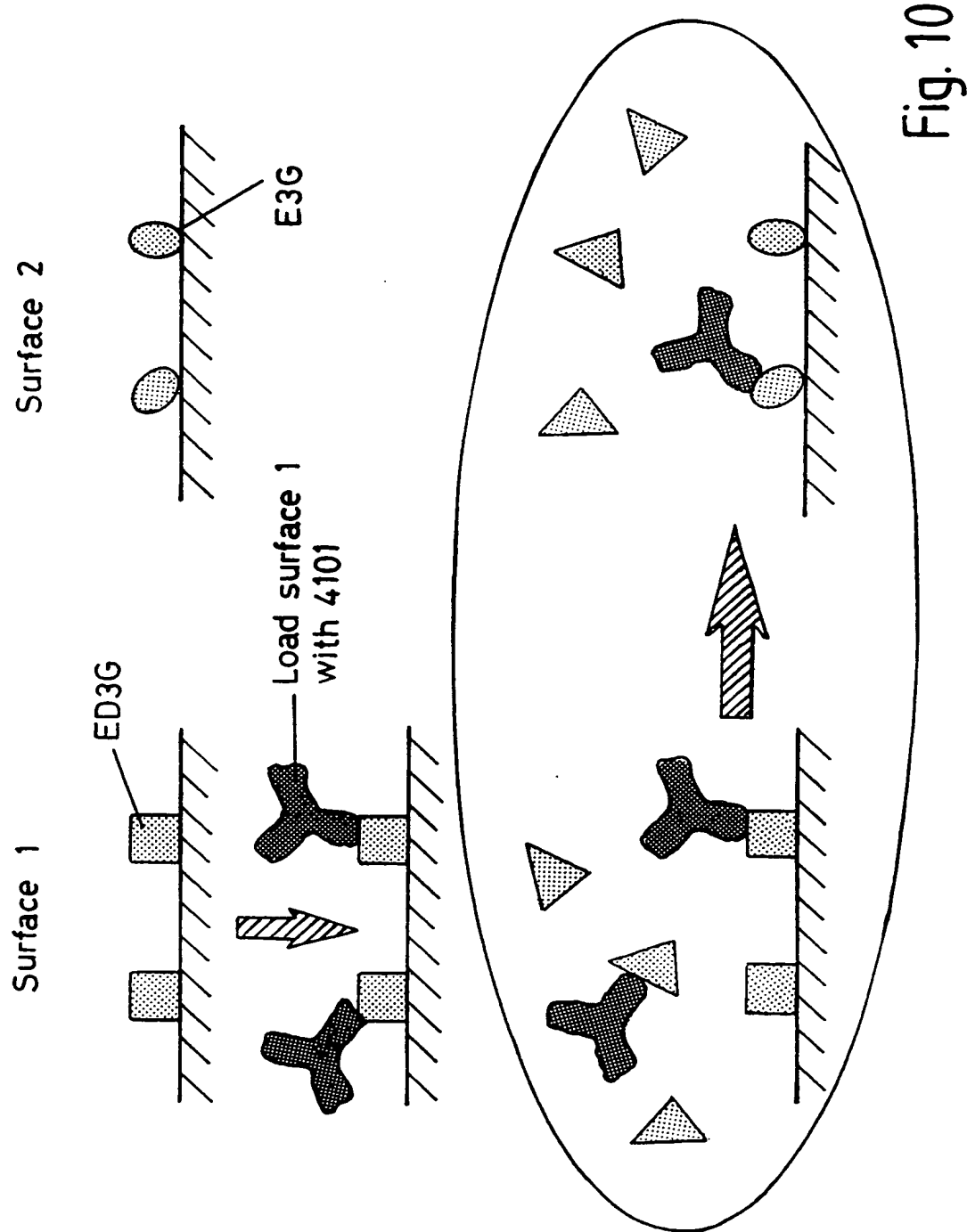


Fig. 10

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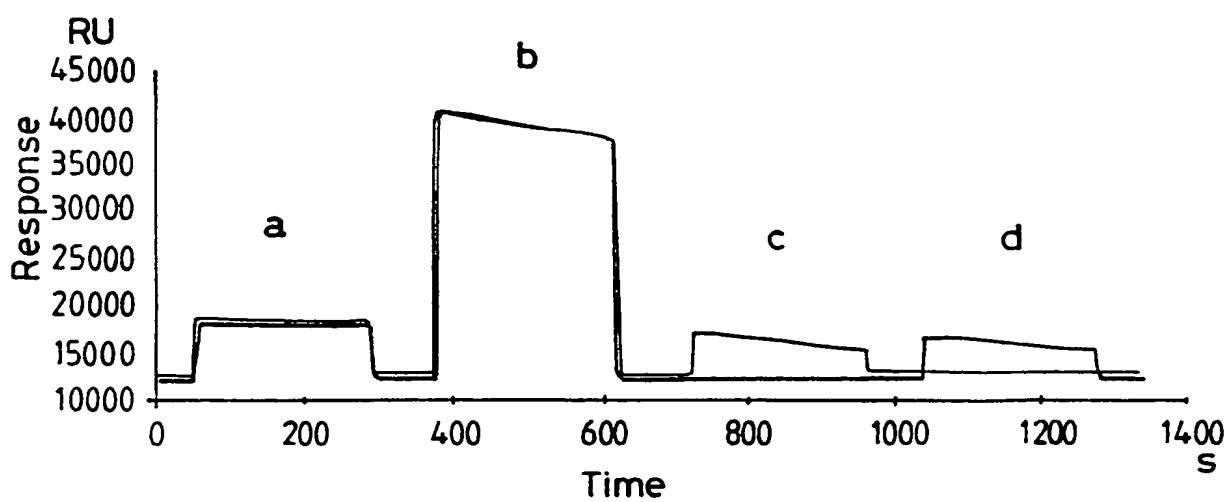


Fig. 11

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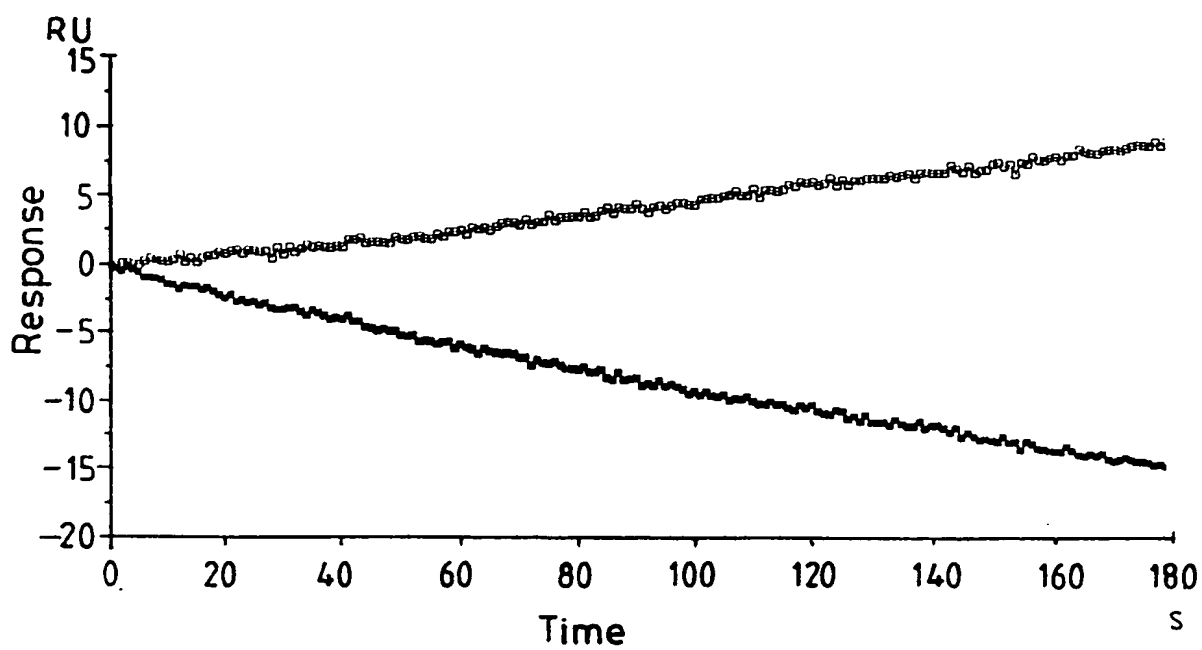


Fig. 12

## SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Unilever PLC  
(B) STREET: Unilever House, Blackfriars  
(C) CITY: London  
(E) COUNTRY: United Kingdom  
(F) POSTAL CODE (ZIP): EC4P 4BQ  
(G) TELEPHONE: (01234) 222068  
(H) TELEFAX: (01234) 222633

(A) NAME: Unilever NV  
(B) STREET: Weena 455  
(C) CITY: Rotterdam  
(E) COUNTRY: Netherlands  
(F) POSTAL CODE (ZIP): 3013 AL

(ii) TITLE OF INVENTION: Improvements in or Relating to Displacement Assays

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15  
Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr  
20 25

# INTERNATIONAL SEARCH REPORT

Application No  
PCT/GB 98/03483

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 21530 A (MESSERSCHMITT BOELKOW BLOHM ;CAMMANN KARL (DE)) 28 October 1993  see page 19, line 14, paragraph 3 - line 20	1-3,5,8, 10,11, 14-24
Y	see page 23 - page 24; claims 7,9; figure 1; example 6	1,12,13
Y	WO 90 06503 A (ARES SERONO RES & DEV LTD) 14 June 1990 see the whole document	1,12
Y	WO 97 27474 A (YISSUM RES DEV CO ;WILLNER ITAMAR (IL); KATZ EUGENII (IL); COHEN Y) 31 July 1997 see abstract	1,13
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 February 1999

Date of mailing of the international search report

08/03/1999

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Authorized officer

Hart-Davis, J



# INTERNATIONAL SEARCH REPORT

Application No

PCT/GB 98/03483

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 18867 A (AMERSHAM INT PLC) 29 October 1992 cited in the application see page 2, line 18 - page 3, line 5; examples ---	1-24
A	WO 91 05262 A (UNIV MICHIGAN) 18 April 1991 cited in the application see example 1 ---	1-11, 14-24
P, A	WO 97 44664 A (UNILEVER PLC ; UNILEVER NV (NL)) 27 November 1997 see the whole document ---	1, 7, 9, 21, 22
A	US 5 514 558 A (CERIANI ROBERTO L ET AL) 7 May 1996 see the whole document -----	1, 6

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No

PCT/GB 98/03483

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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			US 5344784 A	06-09-1994
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			NZ 324815 A	23-12-1998
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US 5514558	A	07-05-1996	US 5532135 A	02-07-1996

## INTERNATIONAL SEARCH REPORT

International Application No

/GB 98/03483

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W0 92 18867 A (AMERSHAM INT PLC) 29 October 1992 cited in the application see page 2, line 18 - page 3, line 5; examples ---	1-24
A	W0 91 05262 A (UNIV MICHIGAN) 18 April 1991 cited in the application see example 1 ---	1-11, 14-24
P,A	W0 97 44664 A (UNILEVER PLC ;UNILEVER NV (NL)) 27 November 1997 see the whole document ---	1,7,9, 21,22
A	US 5 514 558 A (CERIANI ROBERTO L ET AL) 7 May 1996 see the whole document -----	1,6

# INTERNATIONAL SEARCH REPORT

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ational Application No

T/GB 98/03483

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			AU	4755690 A	26-06-1990
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			JP	5504830 T	22-07-1993
			US	5344784 A	06-09-1994
WO 9727474	A	31-07-1997	AU	1207197 A	20-08-1997
			CA	2244214 A	31-07-1997
			EP	0876602 A	11-11-1998
			NZ	324815 A	23-12-1998
WO 9218867	A	29-10-1992	NONE		
WO 9105262	A	18-04-1991	AU	6753390 A	28-04-1991
			US	5281539 A	25-01-1994
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US 5514558	A	07-05-1996	US	5532135 A	02-07-1996